124 Research papers supporting Vaccine/Autism Causation
Ginger Taylor, MS

1. Increased risk of developmental neurologic impairment after high exposure to thimerosal-containing vaccine in first month of life.

   Division of Epidemiology and Surveillance, Vaccine Safety and Development Branch, National Immunization Program, Centers for Disease Control and Prevention. 1999.

   Thomas M. Verstraeten, R. Davies, D. Gu, F DeStefano

   Background: Concern has risen on the presence of the ethylmercury containing preservative thimerosal in vaccines. We assessed the risk for neurologic and renal impairment associated with past exposure to thimerosal-containing vaccine using automated data from the Vaccine Safety Data link (VSD). VSD is a large linked database from four health maintenance organizations in Washington, Oregon and California, containing immunization, medical visit and demographic data on over 400,000 infants born between '91 and '97.

   Methods: We categorized the cumulative ethylmercury exposure from Thimerosal containing vaccines after one month of life and assessed the subsequent risk of degenerative and developmental neurologic disorders and renal disorders before the age of six. We applied proportional hazard models adjusting for HMO, year of birth, and gender, excluding premature babies.

   Results: We identified 286 children with degenerative and 3702 with developmental neurologic disorders, and 310 with renal disorders. The relative risk (RR) of developing a neurologic development disorder was 1.8 (95% confidence intervals [CI] =1.1-2.8) when comparing the highest exposure group at 1 month of age (cumulative dose> 25 ug) to the unexposed group. **Within this group we also found an elevated risk for the following disorders: autism (RR 7.6, 95% CI = 1.8-31.5)**, non organic sleep disorders (RR 5.0, 95% CI = 1.6-15.9}, and speech disorders (RR 2.1, 95% (1=1.1-4.0). For the neurologic degenerative and renal disorders group we found no significantly increased risk or a decreased risk.

   Conclusion: **This analysis suggests that high exposure to ethyl mercury from thimerosal-containing vaccines in the first month of life increases the risk of subsequent development of neurologic development impairment**, but not of neurologic degenerative or renal impairment. Further confirmatory studies are needed.

2. Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997-2002.

   Gallagher CM, Goodman MS.

J Toxicol Environ Health A. 2010;73(24):1665-77. doi: 10.1080/15287394.2010.519317.

Abstract
Universal hepatitis B vaccination was recommended for U.S. newborns in 1991; however, safety findings are mixed. The association between hepatitis B vaccination of male neonates and parental report of autism diagnosis was determined. This cross-sectional study used weighted probability samples obtained from National Health Interview Survey 1997-2002 data sets. Vaccination status was determined from the vaccination record. Logistic regression was used to estimate the odds for autism diagnosis associated with neonatal hepatitis B vaccination among boys age 3-17 years, born before 1999, adjusted for race, maternal education, and two-parent household. **Boys vaccinated as neonates had threefold greater odds for autism diagnosis compared to boys never vaccinated or vaccinated after the first month of life.** Non-Hispanic white boys were 64% less likely to have autism diagnosis relative to nonwhite boys. Findings suggest that U.S. male neonates vaccinated with the hepatitis B vaccine prior to 1999 (from vaccination record) had a threefold higher risk for parental report of autism diagnosis compared to boys not vaccinated as neonates during that same time period. Nonwhite boys bore a greater risk.

3. Gender-selective toxicity of thimerosal.

Exp Toxicol Pathol. 2009 Mar;61(2):133-6. doi: 10.1016/j.etp.2008.07.002. Epub 2008 Sep 3.

Branch DR

Departments of Medicine and Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada. don.branch@utoronto.ca

Abstract
A recent report shows a correlation of the historical use of thimerosal in therapeutic immunizations with the subsequent development of autism; however, this association remains controversial. Autism occurs approximately four times more frequently in males compared to females; thus, studies of thimerosal toxicity should take into consideration gender-selective effects. The present study was originally undertaken to determine the maximum tolerated dose (MTD) of thimersosal in male and female CD1 mice. However, during the limited MTD studies, it became apparent that thimerosal has a differential MTD that depends on whether the mouse is male or female. **At doses of 38.4-76.8mg/kg using 10% DMSO as diluent, seven of seven male mice compared to zero of seven female mice tested succumbed to thimerosal.** Although the thimerosal levels used were very high, as we were originally only trying to determine MTD, it was completely unexpected to observe a difference of the MTD between male and female mice. Thus, our studies, although not directly addressing the controversy surrounding thimerosal and autism, and still preliminary due to small

numbers of mice examined, provide, nevertheless, the first report of gender-selective toxicity of thimerosal and indicate that any future studies of thimerosal toxicity should take into consideration gender-specific differences.

4. Mercury toxicokinetics--dependency on strain and gender.

Toxicol Appl Pharmacol. 2010 Mar 15;243(3):283-91. doi: 10.1016/j.taap.2009.08.026. Epub 2009 Sep 2.

Ekstrand J1, Nielsen JB, Havarinasab S, Zalups RK, Söderkvist P, Hultman P. Molecular and Immunological Pathology, Department of Clinical and Experimental Medicine, Linköping University, SE-58185 Linköping, Sweden.

Abstract
Mercury (Hg) exposure from dental amalgam fillings and thimerosal in vaccines is not a major health hazard, but adverse health effects cannot be ruled out in a small and more susceptible part of the exposed population. Individual differences in toxicokinetics may explain susceptibility to mercury. Inbred, H-2-congenic A.SW and B10.S mice and their F1- and F2-hybrids were given HgCl2 with 2.0 mg Hg/L drinking water and traces of (203)Hg. Whole-body retention (WBR) was monitored until steady state after 5 weeks, when the organ Hg content was assessed. Despite similar Hg intake, **A.SW males attained a 20-30% significantly higher WBR and 2- to 5-fold higher total renal Hg retention/concentration than A.SW females and B10.S mice. A selective renal Hg accumulation but of lower magnitude was seen also in B10.S males compared with females.** Differences in WBR and organ Hg accumulation are therefore regulated by non-H-2 genes and gender. Lymph nodes lacked the strain- and gender-dependent Hg accumulation profile of kidney, liver and spleen. After 15 days without Hg A.SW mice showed a 4-fold higher WBR and liver Hg concentration, but 11-fold higher renal Hg concentration, showing the key role for the kidneys in explaining the slower Hg elimination in A.SW mice. The trait causing higher mercury accumulation was not dominantly inherited in the F1 hybrids. F2 mice showed a large inter-individual variation in Hg accumulation, showing that multiple genetic factors influence the Hg toxicokinetics in the mouse. The genetically heterogeneous human population may therefore show a large variation in mercury toxicokinetics.

5. A Review of the Differences in Developmental, Psychiatric, and Medical Endophenotypes Between Males and Females with Autism Spectrum Disorder

J Dev Phys Disabil. 2015 Feb; 27(1): 119–139.

Eric Rubenstein, Lisa D. Wiggins, and Li-Ching Lee

Eric Rubenstein, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Room E6032, Baltimore, MD 21205, USA;

Contributor Information, Li-Ching Lee: llee38@jhu.edu

Abstract
Autism spectrum disorder (ASD) is over four times more prevalent in males compared to females. Increased understanding of sex differences in ASD endophenotypes could add insight into possible etiologies and the assessment and management of the disorder. Consequently, the purpose of this review is to describe current literature regarding sex differences in the developmental, psychiatric, and medical endophenotypes of ASD in order to illustrate current knowledge and areas in need of further research. Our review found that repetitive behaviors and restricted interests are more common in males than females with ASD. Intellectual disability is more common in females than males with ASD. Attention to detail may be more common in males than females with ASD and epilepsy may be more common in females than males with ASD, although limited research in these areas prevent definitive conclusions from being drawn. There does not appear to be a sex difference in other developmental, psychiatric, and medical symptoms associated with ASD, or the research was contradictory or too sparse to establish a sex difference. Our review is unique in that it offers detailed discussion of sex differences in three major endophenotypes of ASD. Further research is needed to better understand why sex differences exist in certain ASD traits and to evaluate whether phenotypic sex differences are related to different pathways of development, assessment, and treatment of the disorder.

6. Do aluminum vaccine adjuvants contribute to the rising prevalence of autism?

J Inorg Biochem. 2011 Nov;105(11):1489-99. Epub 2011 Aug 23.
Tomljenovic L, Shaw CA.

Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia, 828 W. 10th Ave, Vancouver, BC, Canada V5Z 1L8.

Abstract
Autism spectrum disorders (ASD) are serious multisystem developmental disorders and an urgent global public health concern. Dysfunctional immunity and impaired brain function are core deficits in ASD. Aluminum (Al), the most commonly used vaccine adjuvant, is a demonstrated neurotoxin and a strong immune stimulator. Hence, adjuvant Al has the potential to induce neuroimmune disorders. When assessing adjuvant toxicity in children, two key points ought to be considered: (i) children should not be viewed as "small adults" as their unique physiology makes them much more vulnerable to toxic insults; and (ii) if exposure to Al from only few vaccines can lead to cognitive impairment and autoimmunity in adults, is it unreasonable to question whether the current pediatric schedules, often containing 18 Al adjuvanted vaccines, are safe for children? By applying Hill's criteria for establishing causality between exposure and outcome we investigated whether exposure to Al from vaccines could be contributing to the rise in ASD prevalence in the Western world. Our results show that: (i) children from countries with the highest ASD prevalence appear to have the highest exposure to Al from vaccines; (ii) the increase in exposure to Al adjuvants

significantly correlates with the increase in ASD prevalence in the United States observed over the last two decades (Pearson r=0.92, p<0.0001); and (iii) a significant correlation exists between the amounts of Al administered to preschool children and the current prevalence of ASD in seven Western countries, particularly at 3-4months of age (Pearson r=0.89-0.94, p=0.0018-0.0248). **The application of the Hill's criteria to these data indicates that the correlation between Al in vaccines and ASD may be causal.** Because children represent a fraction of the population most at risk for complications following exposure to Al, a more rigorous evaluation of Al adjuvant safety seems warranted.

7. Administration of aluminium to neonatal mice in vaccine-relevant amounts is associated with adverse long term neurological outcomes.

J Inorg Biochem. 2013 Nov;128:237-44. doi: 10.1016/j.jinorgbio.2013.07.022. Epub 2013 Jul 19.

Shaw CA, Li Y, Tomljenovic L.

Dept. of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, British Columbia, Canada; Program in Experimental Medicine, University of British Columbia, Vancouver, British Columbia, Canada; Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada. Electronic address: cashawlab@gmail.com.

Abstract
Our previous ecological studies of autism spectrum disorder (ASD) has demonstrated a correlation between increasing ASD rates and aluminium (Al) adjuvants in common use in paediatric vaccines in several Western countries. The correlation between ASD rate and Al adjuvant amounts appears to be dose-dependent and satisfies 8 of 9 Hill criteria for causality. We have now sought to provide an animal model to explore potential behavioural phenotypes and central nervous system (CNS) alterations using s.c. injections of Al hydroxide in early postnatal CD-1 mice of both sexes. Injections of a "high" and "low" Al adjuvant levels were designed to correlate to either the U.S. or Scandinavian paediatric vaccine schedules vs. control saline-injected mice. Both male and female mice in the "high Al" group showed significant weight gains following treatment up to sacrifice at 6 months of age. Male mice in the "high Al" group showed significant changes in light-dark box tests and in various measures of behaviour in an open field. Female mice showed significant changes in the light-dark box at both doses, but no significant changes in open field behaviours. These current data implicate Al injected in early postnatal life in some CNS alterations that may be relevant for a better understanding of the aetiology of ASD.

Repetitive administration of aluminium to neonatal mice in amounts comparable to those to children receive via routine vaccinations significantly increases anxiety and reduces exploratory behaviour and locomotor activities. The neurodisruptive effects of aluminium are long-lasting and persist for 6 months following injection.

8. Aluminum-Induced Entropy in Biological Systems: Implications for Neurological Disease

Journal of Toxicology, Volume 2014 (2014), Article ID 491316, 27 pages

Christopher A. Shaw,[1,2,3] Stephanie Seneff,[4] Stephen D. Kette,[5] Lucija Tomljenovic,[1] John W. Oller Jr.,[6] and Robert M. Davidson[7]

[1]Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, 828 W. 10th Avenue, Vancouver, British Columbia, Canada V5Z 1L8
[2]Program Experimental Medicine, University of British Columbia, Vancouver, Canada V5Z 1L8
[3]Program in Neurosciences, University of British Columbia, Vancouver, Canada V5Z 1L8
[4]MIT Computer Science and Artificial Intelligence Laboratory, 32 Vassar Street, Cambridge, MA 02139, USA
[5]Hudson, FL 34667, USA
[6]Department of Communicative Disorders, University of Louisiana, Lafayette, LA 70504-3170, USA
[7]Internal Medicine Group Practice, PhyNet Inc., 4002 Technology Center, Longview, TX 75605, USA

Over the last 200 years, mining, smelting, and refining of aluminum (Al) in various forms have increasingly exposed living species to this naturally abundant metal. Because of its prevalence in the earth’s crust, prior to its recent uses it was regarded as inert and therefore harmless. However, Al is invariably toxic to living systems and has no known beneficial role in any biological systems. Humans are increasingly exposed to Al from food, water, medicinals, vaccines, and cosmetics, as well as from industrial occupational exposure. Al disrupts biological self-ordering, energy transduction, and signaling systems, thus increasing biosemiotic entropy. Beginning with the biophysics of water, disruption progresses through the macromolecules that are crucial to living processes (DNAs, RNAs, proteoglycans, and proteins). It injures cells, circuits, and subsystems and can cause catastrophic failures ending in death. Al forms toxic complexes with other elements, such as fluorine, and interacts negatively with mercury, lead, and glyphosate. Al negatively impacts the central nervous system in all species that have been studied, including humans. Because of the global impacts of Al on water dynamics and biosemiotic systems, CNS disorders in humans are sensitive indicators of the Al toxicants to which we are being exposed.

Exerpts: **"Animal models of neurological disease plainly suggest that the ubiquitous presence of Al in human beings implicates Al toxicants as causally involved in Lou Gehrig’s disease (ALS), Alzheimer’s disease and autism spectrum disorders."**

"All these findings plausibly implicate Al adjuvants in pediatric vaccines as causal factors contributing to increased rates of autism spectrum

disorders in countries where multiple doses are almost universally administered."

9. A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors

Environ Health. 2014; 13: 73.

Cynthia D Nevison

Institute for Arctic and Alpine Research, University of Colorado, Boulder, Boulder, CO 80309-0450 USA

The prevalence of diagnosed autism has increased rapidly over the last several decades among U.S. children. Environmental factors are thought to be driving this increase and a list of the top ten suspected environmental toxins was published recently.

Methods

Temporal trends in autism for birth years 1970–2005 were derived from a combination of data from the California Department of Developmental Services (CDDS) and the United States Individuals with Disabilities Education Act (IDEA). Temporal trends in suspected toxins were derived from data compiled during an extensive literature survey. Toxin and autism trends were compared by visual inspection and computed correlation coefficients. Using IDEA data, autism prevalence vs. birth year trends were calculated independently from snapshots of data from the most recent annual report, and by tracking prevalence at a constant age over many years of reports. The ratio of the snapshot:tracking trend slopes was used to estimate the "real" fraction of the increase in autism.

Results
The CDDS and IDEA data sets are qualitatively consistent in suggesting a strong increase in autism prevalence over recent decades. The quantitative comparison of IDEA snapshot and constant-age tracking trend slopes suggests that ~75-80% of the tracked increase in autism since 1988 is due to an actual increase in the disorder rather than to changing diagnostic criteria. Most of the suspected environmental toxins examined have flat or decreasing temporal trends that correlate poorly to the rise in autism. Some, including lead, organochlorine pesticides and vehicular emissions, have strongly decreasing trends. **Among the suspected toxins surveyed, polybrominated diphenyl ethers, aluminum adjuvants, and the herbicide glyphosate have increasing trends that correlate positively to the rise in autism.**

Conclusions
Diagnosed autism prevalence has risen dramatically in the U.S over the last several decades and continued to trend upward as of birth year 2005. The increase is mainly real and has occurred mostly since the late 1980s. In contrast,

children's exposure to most of the top ten toxic compounds has remained flat or decreased over this same time frame. Environmental factors with increasing temporal trends can help suggest hypotheses for drivers of autism that merit further investigation.

10. Autism: a form of lead and mercury toxicity

    Environ Toxicol Pharmacol. 2014 Nov;38(3):1016-24. doi: 10.1016/j.etap.2014.10.005. Epub 2014 Nov 6.

    Yassa HA

    Abstract

    AIM: Autism is a developmental disability characterized by severe deficits in social interaction and communication. The definite cause of autism is still unknown. The aim of this study is to find out the relation between exposure to Lead and/or mercury as heavy metals and autistic symptoms, dealing with the heavy metals with chelating agents can improve the autistic symptoms.

    METHOD: Blood and hair samples were obtained from 45 children from Upper Egypt with autism between the ages of 2 and 10 years and 45 children served as controls in the same age range, after taken an informed consent and fill a questionnaire to assess the risk factors. The samples were analyzed blindly for lead and mercury by using atomic absorption and ICP-MS. Data from the two groups were compared, then follow up of the autistic children after treatment with chelating agents were done.

    RESULTS: The results obtained showed significant difference among the two groups, there was high level of mercury and lead among those kids with autism. Significant decline in the blood level of lead and mercury with the use of DMSA as a chelating agent. In addition, there was decline in the autistic symptoms with the decrease in the lead and mercury level in blood.

    CONCLUSION: **Lead and mercury considered as one of the main causes of autism. Environmental exposure as well as defect in heavy metal metabolism is responsible for the high level of heavy metals. Detoxification by chelating agents had great role in improvement of those kids.**

11. Toxic Metals and Essential Elements in Hair and Severity of Symptoms among Children with Autism

    Maedica (Buchar). 2012 Jan; 7(1): 38–48.

Eleonor BLAUROCK-BUSCH,a Omnia R. AMIN,b Hani H. DESSOKI,c and Thanaa RABAH d
aLecturer and Advisor, International Board of Clinical Metal Toxicology & German Medical Association of Clinical Metal Toxicology, Hersbruck, Germany
bAssociate Professor of Psychiatry, Cairo University, Egypt
cAssociate Professor of Psychiatry, Beni-Suef University, Egypt - Beni-Suef University
dResearcher of Public Health and Biostatistics, National Research Center, Egypt
Address for correspondence: Eleonor Blaurock-Busch, Laboratory for Clinical and Environmental Analyses. Robenstr 20, D-912217, Hersbruck, Germania. Phone: +0049 91514332 ; Email: ed.ecartorcim@bbew

ABSTRACT

Objective: The objective of this study was to assess the levels of ten toxic metals and essential elements in hair samples of children with autism, and to correlate the level of these elements with the severity of autism.

Method: The participants were 44 children, age 3 to 9 years, with Autistic Spectrum Disorder (ASD) according to Diagnostic and Statistical Manual of Mental Disorders 4th Edition, (DSM-IV). The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS). Hair analysis was performed to evaluate the long term metal exposure and mineral level.

Results: By comparing hair concentration of autistic vs nonautistic children, elevated hair concentrations were noted for aluminum, arsenic, cadmium, mercury, antimony, nickel, lead, and vanadium. Hair levels of calcium, iron, iodine, magnesium, manganese, molybdenum, zinc, and selenium were considered deficient. There was a significant positive correlation between lead & verbal communication ($p = 0.020$) and general impression ($p = 0.008$). In addition, there was a significant negative correlation between zinc & fear and nervousness ($p = 0.022$).

Conclusion: Our data supports the historic evidence that heavy metals play a role in the development of ASD. In combination with an inadequate nutritional status the toxic effect of metals increase along with the severity of symptoms.

12. Assessment of infantile mineral imbalances in autism spectrum disorders (ASDs).

Int J Environ Res Public Health. 2013 Nov 11;10(11):6027-43. doi: 10.3390/ijerph10116027.

Yasuda H1, Tsutsui T.

La Belle Vie Research Laboratory, 8-4 Nihonbashi-Tomizawacho, Chuo-ku, Tokyo 103-0006, Japan. yasuda@lbv.co.jp

Abstract
The interactions between genes and the environment are now regarded as the most probable explanation for autism. In this review, we summarize the results of a metallomics study in which scalp hair concentrations of 26 trace elements were examined for 1,967 autistic children (1,553 males and 414 females aged 0-15 years-old), and discuss recent advances in our understanding of epigenetic roles of infantile mineral imbalances in the pathogenesis of autism. In the 1,967 subjects, 584 (29.7%) and 347 (17.6%) were found deficient in zinc and magnesium, respectively, and the incidence rate of zinc deficiency was estimated at 43.5% in male and 52.5% in female infantile subjects aged 0-3 years-old. In contrast, 339 (17.2%), 168 (8.5%) and 94 (4.8%) individuals were found to suffer from high burdens of aluminum, cadmium and lead, respectively, and 2.8% or less from mercury and arsenic. High toxic metal burdens were more frequently observed in the infants aged 0-3 years-old, whose incidence rates were 20.6%, 12.1%, 7.5%, 3.2% and 2.3% for aluminum, cadmium, lead, arsenic and mercury, respectively. These findings suggest that infantile zinc- and magnesium-deficiency and/or toxic metal burdens may be critical and induce epigenetic alterations in the genes and genetic regulation mechanisms of neurodevelopment in the autistic children, and demonstrate that a time factor "infantile window" is also critical for neurodevelopment and probably for therapy. Thus, early metallomics analysis may lead to early screening/estimation and treatment/prevention for the autistic neurodevelopment disorders.

13. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism.

J Biomed Sci. 2002 Jul-Aug;9(4):359-64.

Singh VK, Lin SX, Newell E, Nelson C., Department of Biology and Biotechnology Center, Utah State University, Logan, Utah 84322, USA. singhvk@cc.usu.edu

Abstract

Autoimmunity to the central nervous system (CNS), especially to myelin basic protein (MBP), may play a causal role in autism, a neurodevelopmental disorder. Because many autistic children harbor elevated levels of measles antibodies, we conducted a serological study of measles-mumps-rubella (MMR) and MBP autoantibodies. Using serum samples of 125 autistic children and 92 control children, antibodies were assayed by ELISA or immunoblotting methods. ELISA analysis showed a significant increase in the level of MMR antibodies in autistic children. Immunoblotting analysis revealed the presence of an unusual MMR antibody in 75 of 125 (60%) autistic sera but not in control sera. This antibody specifically detected a protein of 73-75 kD of MMR. This protein band, as analyzed with monoclonal antibodies, was immunopositive for measles hemagglutinin (HA) protein but not for measles nucleoprotein and rubella or mumps viral proteins. Thus the MMR antibody in autistic sera detected measles HA protein, which is unique to the measles subunit of the vaccine. Furthermore,

over 90% of MMR antibody-positive autistic sera were also positive for MBP autoantibodies, suggesting a strong association between MMR and CNS autoimmunity in autism. Stemming from this evidence, we suggest that **an inappropriate antibody response to MMR, specifically the measles component thereof, might be related to pathogenesis of autism.**

14. Infection, vaccines and other environmental triggers of autoimmunity.

Autoimmunity. 2005 May;38(3):235-45.

Molina V, Shoenfeld Y., Department of Medicine B and The Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel.

Abstract
The etiology of autoimmune diseases is still not clear but genetic, immunological, hormonal and environmental factors are considered to be important triggers. Most often autoimmunity is not followed by clinical symptoms unless an additional event such as an environmental factor favors an overt expression. Many environmental factors are known to affect the immune system and may play a role as triggers of the autoimmune mosaic.Infections: bacterial, viral and parasitic infections are known to induce and exacerbate autoimmune diseases, mainly by the mechanism of molecular mimicry. This was studied for some syndromes as for the association between SLE and EBV infection, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection and more. Vaccines, in several reports were found to be temporally followed by a new onset of autoimmune diseases. The same mechanisms that act in infectious invasion of the host, apply equally to the host response to vaccination. It has been accepted for diphtheria and tetanus toxoid, polio and measles vaccines and GBS. Also this theory has been accepted for MMR vaccination and development of autoimmune thrombocytopenia, MS has been associated with HBV vaccination. Occupational and other chemical exposures are considered as triggers for autoimmunity. A debate still exists about the role of silicone implants in induction of scleroderma like disease.Not only foreign chemicals and agents have been associated with induction of autoimmunity, but also an intrinsic hormonal exposure, such as estrogens. This might explain the sexual dimorphism in autoimmunity.Better understanding of these environmental risk factors will likely lead to explanation of the mechanisms of onset and progression of autoimmune diseases and may lead to effective preventive involvement in specific high-risk groups. So by diagnosing a new patient with autoimmune disease a wide anamnesis work should be done.

15. Impact of environmental factors on the prevalence of autistic disorder after 1979

Journal of Public Health and Epidemiology, Vol.6(9), pp. 271-284, September 2014

Theresa A. Deisher, Ngoc V. Doan, Angelica Omaiye, Kumiko Koyama, Sarah Bwabye

Abstract
The aim of this study was to investigate a previously overlooked, universally introduced environmental factor, fetal and retroviral contaminants in childhood vaccines, absent prior to change points (CPs) in autistic disorder (AD) prevalence with subsequent dose-effect evidence and known pathologic mechanisms of action. Worldwide population based cohort study was used for the design of this study. The United States, Western Australia, United Kingdom and Denmark settings were used. All live born infants who later developed autistic disorder delivered after 1 January 1970, whose redacted vaccination and autistic disorder diagnosis information is publicly available in databases maintained by the US Federal Government, Western Australia, UK, and Denmark. The live births, grouped by father's age, were from the US and Australia. The children vaccinated with MMRII, Varicella and Hepatitis A vaccines varied from 19 to 35 months of age at the time of vaccination. Autistic disorder birth year change points were identified as 1980.9, 1988.4 and 1996 for the US, 1987 for UK, 1990.4 for Western Australia, and 1987.5 for Denmark. Change points in these countries corresponded to introduction of or increased doses of human fetal cell line-manufactured vaccines, while no relationship was found between paternal age or Diagnostic and Statistical Manual (DSM) revisions and autistic disorder diagnosis. Further, linear regression revealed that Varicella and Hepatitis A immunization coverage was significantly correlated to autistic disorder cases. R software was used to calculate change points. **Autistic disorder change points years are coincident with introduction of vaccines manufactured using human fetal cell lines, containing fetal and retroviral contaminants, into childhood vaccine regimens. This pattern was repeated in the US, UK, Western Australia and Denmark. Thus, rising autistic disorder prevalence is directly related to vaccines manufactured utilizing human fetal cells. Increased paternal age and DSM revisions were not related to rising autistic disorder prevalence.**

16. A Positive Association found between Autism Prevalence and Childhood Vaccination uptake across the U.S. Population

Journal of Toxicology and Environmental Health, Part A: Current Issues
Volume 74, Issue 14, 2011, Pages 903 - 916
Author: Gayle DeLonga

Abstract
The reason for the rapid rise of autism in the United States that began in the 1990s is a mystery. Although individuals probably have a genetic predisposition to develop autism, researchers suspect that one or more environmental triggers are also needed. One of those triggers might be the battery of vaccinations that young children receive. Using regression analysis and controlling for family income and ethnicity, the relationship between the proportion of children who received the recommended vaccines by age 2 years and the prevalence of autism (AUT) or speech or language impairment (SLI) in each U.S. state from

2001 and 2007 was determined. A positive and statistically significant relationship was found: The higher the proportion of children receiving recommended vaccinations, the higher was the prevalence of AUT or SLI. A 1% increase in vaccination was associated with an additional 680 children having AUT or SLI. Neither parental behavior nor access to care affected the results, since vaccination proportions were not significantly related (statistically) to any other disability or to the number of pediatricians in a U.S. state. **The results suggest that although mercury has been removed from many vaccines, other culprits may link vaccines to autism.** Further study into the relationship between vaccines and autism is warranted. To read the abstract click HERE.

17. Neonatal administration of a vaccine preservative, thimerosal, produces lasting impairment of nociception and apparent activation of opioid system in rats.

    Brain Res. 2009 Dec 8;1301:143-51. Epub 2009 Sep 9.

    Olczak M, Duszczyk M, Mierzejewski P, Majewska MD. Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Warsaw, Poland.

    Abstract
    Thimerosal (THIM), an organomercury preservative added to many child vaccines is a suspected factor in pathogenesis of neurodevelopmental disorders. We examined the pharmacokinetics of Hg in the brain, liver and kidneys after i.m. THIM injection in suckling rats and we tested THIM effect on nociception. THIM solutions were injected to Wistar and Lewis rats in a vaccination-like mode on PN days 7, 9, 11 and 15 in four equal doses. For Wistar rats these were: 12, 48, 240, 720, 1440, 2160, 3000 microg Hg/kg and for Lewis: 54, 216, 540 and 1080 microg Hg/kg. Pharmacokinetic analysis revealed that Hg from THIM injections accumulates in the rat brain in significant amounts and remains there longer than 30 days after the injection. At the 6th week of age animals were examined for pain sensitivity using the hot plate test. THIM treated rats of both strains and sexes manifested statistically significantly elevated pain threshold (latency for paw licking, jumping) on a hot plate (56 degrees C). Wistar rats were more sensitive to this effect than Lewis rats. Protracted THIM-induced hypoalgesia was reversed by naloxone (5 mg/kg, i.p.) injected before the hot plate test, indicative of involvement of endogenous opioids. This was confirmed by augmented catalepsy after morphine (2.5 mg/kg, s.c.) injection. Acute THIM injection to 6-week-old rats also produced hypoalgesia, but this effect was transient and was gone within 14 days. **Present findings show that THIM administration to suckling or adult rats impairs sensitivity to pain, apparently due to activation the endogenous opioid system.**

18. Transcriptomic analyses of neurotoxic effects in mouse brain after intermittent neonatal administration of thimerosal.

Toxicol Sci. 2014 Jun;139(2):452-65. doi: 10.1093/toxsci/kfu049. Epub 2014 Mar 27.

State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China.Li X1, Qu F, Xie W, Wang F, Liu H, Song S, Chen T, Zhang Y, Zhu S, Wang Y, Guo C, Tang TS.

Abstract

Thimerosal is a vaccine antimicrobial preservative which has long been suspected an iatrogenic factor possibly contributing to neurodevelopmental disorders including autism. The association between infant vaccine thimerosal exposure and autism remains an open question. Although thimerosal has been removed from mandatory childhood vaccines in the United States, thimerosal-preserved vaccines are still widely used outside of the United States especially in developing countries. Notably, thimerosal-containing vaccines are being given to the newborns within the first 12-24 h after birth in some countries. To examine the possible neurotoxic effects of early neonatal exposure to a higher level of thimerosal, FVB mice were subcutaneously injected with thimerosal-mercury at a dose which is 20× higher than that used for regular Chinese infant immunization during the first 4 months of life. Thimerosal-treated mice exhibited neural development delay, social interaction deficiency, and inclination of depression. Apparent neuropathological changes were also observed in adult mice neonatally treated with thimerosal. High-throughput RNA sequencing of autistic-behaved mice brains revealed the alternation of a number of canonical pathways involving neuronal development, neuronal synaptic function, and the dysregulation of endocrine system. Intriguingly, the elevation of anterior pituitary secreting hormones occurred exclusively in male but not in female thimerosal-treated mice, demonstrating for the first time the gender bias of thimerosal-mercury toxicity with regard to endocrine system. **Our results indicate that higher dose of neonatal thimerosal-mercury (20× higher than that used in human) is capable of inducing long-lasting substantial dysregulation of neurodevelopment, synaptic function, and endocrine system, which could be the causal involvements of autistic-like behavior in mice.**

19. Lasting neuropathological changes in rat brain after intermittent neonatal administration of thimerosal.

Folia Neuropathol. 2010;48(4):258-69. Olczak M, Duszczyk M, Mierzejewski P, Wierzba-Bobrowicz T, Majewska MD.

Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, ul. Sobieskiego 9, Warsaw, Poland.

Abstract
Thimerosal, an organomercurial added as a preservative to some vaccines, is a suspected iatrogenic factor, possibly contributing to paediatric neurodevelopmental disorders including autism. We examined the effects of early

postnatal administration of thimerosal (four i.m. injections, 12 or 240 μg THIM-Hg/kg, on postnatal days 7, 9, 11 and 15) on brain pathology in Wistar rats. Numerous neuropathological changes were observed in young adult rats which were treated postnatally with thimerosal. They included: ischaemic degeneration of neurons and "dark" neurons in the prefrontal and temporal cortex, the hippocampus and the cerebellum, pathological changes of the blood vessels in the temporal cortex, diminished synaptophysin reaction in the hippocampus, atrophy of astroglia in the hippocampus and cerebellum, and positive caspase-3 reaction in Bergmann astroglia. **These findings document neurotoxic effects of thimerosal, at doses equivalent to those used in infant vaccines or higher, in developing rat brain, suggesting likely involvement of this mercurial in neurodevelopmental disorders.**

20. Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats.

Behav Brain Res. 2011 Sep 30;223(1):107-18. doi: 10.1016/j.bbr.2011.04.026. Epub 2011 Apr 28.

Olczak M, Duszczyk M, Mierzejewski P, Meyza K, Majewska MD. Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, 02-957 Warsaw, Poland.

Abstract
The neurotoxic organomercurial thimerosal (THIM), used for decades as vaccine preservative, is a suspected factor in the pathogenesis of some neurodevelopmental disorders. Previously we showed that neonatal administration of THIM at doses equivalent to those used in infant vaccines or higher, causes lasting alterations in the brain opioid system in rats. Here we investigated neonatal treatment with THIM (at doses 12, 240, 1440 and 3000 μg Hg/kg) on behaviors, which are characteristically altered in autism, such as locomotor activity, anxiety, social interactions, spatial learning, and on the brain dopaminergic system in Wistar rats of both sexes. Adult male and female rats, which were exposed to the entire range of THIM doses during the early postnatal life, manifested impairments of locomotor activity and increased anxiety/neophobia in the open field test. In animals of both sexes treated with the highest THIM dose, the frequency of prosocial interactions was reduced, while the frequency of asocial/antisocial interactions was increased in males, but decreased in females. Neonatal THIM treatment did not significantly affect spatial learning and memory. THIM-exposed rats also manifested reduced haloperidol-induced catalepsy, accompanied by a marked decline in the density of striatal D_2 receptors, measured by immunohistochemical staining, suggesting alterations to the brain dopaminergic system. Males were more sensitive than females to some neurodisruptive/neurotoxic actions of THIM. **These data document that early postnatal THIM administration causes lasting neurobehavioral impairments and neurochemical alterations in the brain, dependent on dose and sex. If similar changes occur in THIM/mercurial-exposed children, they could contribute do neurodevelopmental disorders.**

21. B-Lymphocytes from a Population of Children with Autism Spectrum Disorder and Their Unaffected Siblings Exhibit Hypersensitivity to Thimerosal

J Toxicol. 2013;2013:801517. Epub 2013 Jun 9.

Sharpe MA, Gist TL, Baskin DS.

Department of Neurosurgery, The Methodist Neurological Institute, Houston, TX.

Abstract

The role of thimerosal containing vaccines in the development of autism spectrum disorder (ASD) has been an area of intense debate, as has the presence of mercury dental amalgams and fish ingestion by pregnant mothers. We studied the effects of thimerosal on cell proliferation and mitochondrial function from B-lymphocytes taken from individuals with autism, their nonautistic twins, and their nontwin siblings. Eleven families were examined and compared to matched controls. B-cells were grown with increasing levels of thimerosal, and various assays (LDH, XTT, DCFH, etc.) were performed to examine the effects on cellular proliferation and mitochondrial function. A subpopulation of eight individuals (4 ASD, 2 twins, and 2 siblings) from four of the families showed thimerosal hypersensitivity, whereas none of the control individuals displayed this response. The thimerosal concentration required to inhibit cell proliferation in these individuals was only 40% of controls. Cells hypersensitive to thimerosal also had higher levels of oxidative stress markers, protein carbonyls, and oxidant generation. **This suggests certain individuals with a mild mitochondrial defect may be highly susceptible to mitochondrial specific toxins like the vaccine preservative thimerosal.**

22. Thimerosal-Derived Ethylmercury Is a Mitochondrial Toxin in Human Astrocytes: Possible Role of Fenton Chemistry in the Oxidation and Breakage of mtDNA

J Toxicol. 2012; 2012: 373678. Published online Jun 28, 2012. doi: 10.1155/2012/373678

Martyn A. Sharpe, * Andrew D. Livingston, and David S. Baskin

Abstract
Thimerosal generates ethylmercury in aqueous solution and is widely used as preservative. We have investigated the toxicology of Thimerosal in normal human astrocytes, paying particular attention to mitochondrial function and the generation of specific oxidants. **We find that ethylmercury not only inhibits mitochondrial respiration leading to a drop in the steady state membrane potential, but also concurrent with these phenomena increases the formation of superoxide, hydrogen peroxide, and Fenton/Haber-Weiss**

generated hydroxyl radical. These oxidants increase the levels of cellular aldehyde/ketones. Additionally, we find a five-fold increase in the levels of oxidant damaged mitochondrial DNA bases and increases in the levels of mtDNA nicks and blunt-ended breaks. Highly damaged mitochondria are characterized by having very low membrane potentials, increased superoxide/hydrogen peroxide production, and extensively damaged mtDNA and proteins. These mitochondria appear to have undergone a permeability transition, an observation supported by the five-fold increase in Caspase-3 activity observed after Thimerosal treatment.

23. Thioredoxin: A novel, independent diagnosis marker in children with autism.

Int J Dev Neurosci. 2014 Nov 26. pii: S0736-5748(14)00191-9. doi: 10.1016/j.ijdevneu.2014.11.007.

Zhang QB1, Gao SJ1, Zhao HX2.

Abstract
BACKGROUND:
Oxidative stress increases serum thioredoxin (TRX), a redox-regulating protein with antioxidant activity recognized as an oxidative-stress marker. The aim of this study was to assess the clinical significance of serum TRX levels in Autism spectrum disorders (ASD).
METHODS:
Eighty patients diagnosed with ASD and 100 sex and age matched typically developing children were assessed for serum TRX content at admission. TRX were assayed with solid-phase sandwich ELISA, and severity of ASD was evaluated with the Childhood Autism Rating Scale (CARS) Score.
RESULTS:
The results indicated that the median serum TRX levels were significantly ($P<0.0001$) higher in children with ASD as compared to typically developing children [17.9(IQR: 10.7-25.8)ng/ml and 5.5(3.6-9.2)ng/ml, respectively]. Levels of TRX increased with increasing severity of ASD as defined by the CARS score. After adjusting for all other possible covariates, TRX still was an independent diagnosis marker of ASD with an adjusted OR of 1.454 (95% CI, 1.232-1.892; $P<0.0001$). Based on the receiver operating characteristic (ROC) curve, the optimal cut-off value of serum TRX levels as an indicator for auxiliary diagnosis of autism was projected to be 10.6ng/ml. Further, we found that an increased diagnosis of ASD was associated with TRX levels ≥10.6ng/ml (adjusted OR 15.31, 95% CI: 7.36-31.85) after adjusting for possible confounders.
CONCLUSIONS:
Our study demonstrated that serum TRX levels were associated with ASD, and elevated levels could be considered as a novel, independent diagnosis indicator of ASD.

24. Inhibition of the human thioredoxin system. A molecular mechanism of mercury toxicity.

J Biol Chem. 2008 May 2;283(18):11913-23. doi: 10.1074/jbc.M710133200. Epub 2008 Mar 4.

Carvalho CM1, Chew EH, Hashemy SI, Lu J, Holmgren A.

Abstract
Mercury toxicity mediated by different forms of mercury is a major health problem; however, the molecular mechanisms underlying toxicity remain elusive. We analyzed the effects of mercuric chloride (HgCl(2)) and monomethylmercury (MeHg) on the proteins of the mammalian thioredoxin system, thioredoxin reductase (TrxR) and thioredoxin (Trx), and of the glutaredoxin system, glutathione reductase (GR) and glutaredoxin (Grx). HgCl(2) and MeHg inhibited recombinant rat TrxR with IC(50) values of 7.2 and 19.7 nm, respectively. Fully reduced human Trx1 bound mercury and lost all five free thiols and activity after incubation with HgCl(2) or MeHg, but only HgCl(2) generated dimers. Mass spectra analysis demonstrated binding of 2.5 mol of Hg(2+) and 5 mol of MeHg(+)/mol of Trx1 with the very strong Hg(2+) complexes involving active site and structural disulfides. Inhibition of both TrxR and Trx activity was observed in HeLa and HEK 293 cells treated with HgCl(2) or MeHg. GR was inhibited by HgCl(2) and MeHg in vitro, but no decrease in GR activity was detected in cell extracts treated with mercurials. Human Grx1 showed similar reactivity as Trx1 with both mercurial compounds, with the loss of all free thiols and Grx dimerization in the presence of HgCl(2), but no inhibition of Grx activity was observed in lysates of HeLa cells exposed to mercury. Overall, mercury inhibition was selective toward the thioredoxin system. In particular, the remarkable potency of the mercury compounds to bind to the selenol-thiol in the active site of TrxR should be a major molecular mechanism of mercury toxicity.

25. Effects of selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: implications for treatment of mercury poisoning.

FASEB J. 2011 Jan;25(1):370-81. doi: 10.1096/fj.10-157594. Epub 2010 Sep 1.

Carvalho CM1, Lu J, Zhang X, Arnér ES, Holmgren A.

Abstract
Mercury toxicity is a highly interesting topic in biomedicine due to the severe endpoints and treatment limitations. Selenite serves as an antagonist of mercury toxicity, but the molecular mechanism of detoxification is not clear. Inhibition of the selenoenzyme thioredoxin reductase (TrxR) is a suggested mechanism of toxicity. Here, we demonstrated enhanced inhibition of activity by inorganic and organic mercury compounds in NADPH-reduced TrxR, consistent with binding of mercury also to the active site selenolthiol. On treatment with 5 μM selenite and NADPH, TrxR inactivated by HgCl(2) displayed almost full recovery of activity. Structural analysis indicated that mercury was complexed with TrxR, but enzyme-generated selenide removed mercury as mercury selenide, regenerating the active site selenocysteine and cysteine residues required for activity. The antagonistic effects on TrxR inhibition were extended to endogenous

antioxidants, such as GSH, and clinically used exogenous chelating agents BAL, DMPS, DMSA, and α-lipoic acid. Consistent with the in vitro results, recovery of TrxR activity and cell viability by selenite was observed in HgCl(2)-treated HEK 293 cells. These results stress the role of TrxR as a target of mercurials and provide the mechanism of selenite as a detoxification agent for mercury poisoning.

26. Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism.

Clin Immunol Immunopathol. 1998 Oct;89(1):105-8.

Singh VK, Lin SX, Yang VC. College of Pharmacy, University of Michigan, Ann Arbor, Michigan, 48109-1065, USA.

Abstract
Considering an autoimmunity and autism connection, brain autoantibodies to myelin basic protein (anti-MBP) and neuron-axon filament protein (anti-NAFP) have been found in autistic children. In this current study, we examined associations between virus serology and autoantibody by simultaneous analysis of measles virus antibody (measles-IgG), human herpesvirus-6 antibody (HHV-6-IgG), anti-MBP, and anti-NAFP. We found that measles-IgG and HHV-6-IgG titers were moderately higher in autistic children but they did not significantly differ from normal controls. Moreover, we found that a vast majority of virus serology-positive autistic sera was also positive for brain autoantibody: (i) 90% of measles-IgG-positive autistic sera was also positive for anti-MBP; (ii) 73% of measles-IgG-positive autistic sera was also positive for anti-NAFP; (iii) 84% of HHV-6-IgG-positive autistic sera was also positive for anti-MBP; and (iv) 72% of HHV-6-IgG-positive autistic sera was also positive for anti-NAFP. **This study is the first to report an association between virus serology and brain autoantibody in autism; it supports the hypothesis that a virus-induced autoimmune response may play a causal role in autism.**

27. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism

American Journal of Clinical Nutrition, Vol. 80, No. 6, 1611-1617, December 2004

Department of Pediatrics, University of Arkansas for Medical Sciences, and the Arkansas Children's Hospital Research Institute

Abstract
Background: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and

environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism.

Objective: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism.

Design: Plasma concentrations of methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folinic acid, betaine, and methylcobalamin was initiated in a subset of the autistic children.

Results: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children.

Conclusions: **An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism**.

28. Porphyrinuria in childhood autistic disorder: Implications for environmental toxicity

Toxicology and Applied Pharmacology, 2006

Robert Natafa, Corinne Skorupkab, Lorene Ametb, Alain Lama, Anthea Springbettc and Richard Lathed, aLaboratoire Philippe Auguste, Paris, France, Association ARIANE, Clichy, France, Department of Statistics, Roslin Institute, Roslin, UK, Pieta Research,

This new study from France utilizes a new and sophisticated measurement for environmental toxicity by assessing porphyrin levels in autistic children. It provides clear and unequivocal evidence that children with autism spectrum disorders are more toxic than their neurotypical peers.

Excerpt: "Coproporphyrin levels were elevated in children with autistic disorder relative to control groups...the elevation was significant. These data implicate environmental toxicity in childhood autistic disorder."

Abstract

To address a possible environmental contribution to autism, we carried out a retrospective study on urinary porphyrin levels, a biomarker of environmental toxicity, in 269 children with neurodevelopmental and related disorders referred to a Paris clinic (2002–2004), including 106 with autistic disorder. Urinary porphyrin levels determined by high-performance liquid chromatography were compared between diagnostic groups including internal and external control groups. Coproporphyrin levels were elevated in children with autistic disorder relative to control groups. Elevation was maintained on normalization for age or to a control heme pathway metabolite (uroporphyrin) in the same samples. The elevation was significant ($P < 0.001$). Porphyrin levels were unchanged in Asperger's disorder, distinguishing it from autistic disorder. The atypical molecule precoproporphyrin, a specific indicator of heavy metal toxicity, was also elevated in autistic disorder ($P < 0.001$) but not significantly in Asperger's. A subgroup with autistic disorder was treated with oral dimercaptosuccinic acid (DMSA) with a view to heavy metal removal. Following DMSA there was a significant ($P = 0.002$) drop in urinary porphyrin excretion. **These data implicate environmental toxicity in childhood autistic disorder.**

29. An investigation of porphyrinuria in Australian children with autism.

J Toxicol Environ Health A. 2008;71(20):1349-51. doi: 10.1080/15287390802271723.

Austin DW, Shandley K.

Swinburne Autism Bio-Research Initiative (SABRI), Faculty of Life and Social Sciences, Swinburne University of Technology, Melbourne, Australia. daustin@swin.edu.au

Abstract

Two recent studies, from France (Nataf et al., 2006) and the United States (Geier & Geier, 2007), identified atypical urinary porphyrin profiles in children with an autism spectrum disorder (ASD). These profiles serve as an indirect measure of environmental toxicity generally, and mercury (Hg) toxicity specifically, with the latter being a variable proposed as a causal mechanism of ASD (Bernard et al., 2001; Mutter et al., 2005). To examine whether this phenomenon occurred in a sample of Australian children with ASD, an analysis of urinary porphyrin profiles was conducted. A consistent trend in abnormal porphyrin levels was evidenced when data was compared with those previously reported in the literature. The results are suggestive of environmental toxic exposure impairing heme synthesis. **Three independent studies from three continents have now demonstrated that porphyrinuria is concomitant with ASD, and that Hg may be a likely xenobiotic to produce porphyrin profiles of this nature.**

30. Porphyrinuria in Korean children with autism: correlation with oxidative stress.

J Toxicol Environ Health A. 2010;73(10):701-10. doi: 10.1080/15287391003614000.

Youn SI1, Jin SH, Kim SH, Lim S.

Department of Basic Eastern Medical Science, Graduate School, KyungHee University, Seoul, Republic of Korea.

Abstract
Autism spectrum disorder (ASD) is a neurodevelopmental disorder believed to be associated with heavy metal exposure, especially mercury (Hg), and is characterized by disturbances in metal elimination. Various studies correlated elevated heavy metal body burden with ASD diagnoses as evidenced by increased urinary porphyrin levels in patients. Urinary porphyrins were also determined in Korean patients diagnosed with ASD (n = 65) who visited AK Eastern Medicinal Clinic in Kangnam-gu, Seoul, from June 2007 to September 2008, compared to controls (n = 9) residing in the same area, by means of Metametrix (CLIA-approved) laboratory testing. Further, urinary organic acids as indicators of hepatic detoxification/oxidative stress were also analyzed among patients diagnosed with ASD. Significant increases were found in patients diagnosed with ASD for proporphyrins, pentacarboxyporphyrin, precoproporphyrin, coproporphyrins, and total porphyrins. Significant correlations were observed between hepatic detoxification/oxidative stress markers and urinary porphyrins. **In agreement with published data, the present results demonstrated that measurement of porphyrins serves as a reliable tool for diagnosis of heavy metal involvement in ASD.**

31. Uncoupling of ATP-mediated Calcium Signaling and Dysregulated IL-6 Secretion in Dendritic Cells by Nanomolar Thimerosal

Environmental Health Perspectives, July 2006.

Samuel R. Goth, Ruth A. Chu Jeffrey P. Gregg

1 National Institute of Environmental Health Sciences Center for Children’s Environmental Health
2 Department of Veterinary Molecular Biosciences and
3 Department of Medical Pathology, University of California–Davis, Davis, California, USA
4 MIND (Medical Investigation of Neurodevelopmental Disorders) Institute, University of California–Davis, Sacramento, California, USA

Address correspondence to I.N. Pessah, Department of Veterinary Medicine, Molecular Biosciences, 1311 Haring Hall, One Shields Ave., University of California, Davis, CA

Abstract
Dendritic cells (DCs), a rare cell type widely distributed in the soma, are potent antigen-presenting cells that initiate primary immune responses. DCs rely on intracellular redox state and calcium (Ca2+) signals for proper development and function, but the relationship between these two signaling systems is unclear. Thimerosal (THI) is a mercurial used to preserve vaccines and consumer products, and is used experimentally to induce Ca2+ release from microsomal stores. We tested adenosine triphosphate (ATP)-mediated Ca2+ responses of DCs transiently exposed to nanomolar THI. Transcriptional and immunocytochemical analyses show that murine myeloid immature DCs (IDCs) and mature DCs (MDCs) express inositol 1,4,5-trisphosphate receptor (IP3R) and ryanodine receptor (RyR) Ca2+ channels, known targets of THI. IDCs express the RyR1 isoform in a punctate distribution that is densest near plasma membranes and within dendritic processes, whereas IP3Rs are more generally distributed. RyR1 positively and negatively regulates purinergic signaling because ryanodine (Ry) blockade a) recruited 80% more ATP responders, b) shortened ATP-mediated Ca2+ transients > 2-fold, and c) produced a delayed and persistent rise (≥ 2-fold) in baseline Ca2+. THI (100 nM, 5 min) recruited more ATP responders, shortened the ATP-mediated Ca2+ transient (≥ 1.4-fold), and produced a delayed rise (≥ 3-fold) in the Ca2+ baseline, mimicking Ry. THI and Ry, in combination, produced additive effects leading to uncoupling of IP3R and RyR1 signals. THI altered ATP-mediated interleukin-6 secretion, initially enhancing the rate of cytokine secretion but suppressing cytokine secretion overall in Dcs. **Dendritic cells are exquisitely sensitive to Thimerosal, with one mechanism involving the uncoupling of positive and negative regulation of Ca2+ signals contributed by RyR1.**

Excerpt: "Our findings that DCs primarily express the RyR1 channel complex and that this complex is uncoupled by very low levels of THI with dysregulated IL-6 secretion raise intriguing questions about a molecular basis for immune dyregulation and the possible role of the RyR1 complex in genetic susceptibility of the immune system to mercury."

32. Myeloid dendritic cells frequencies are increased in children with autism spectrum disorder and associated with amygdala volume and repetitive behaviors

Brain, Behavior, and Immunity, Volume 31, July 2013, Pages 69–75, Inflammation and Mental Health

Elizabeth Breecea, b, Brian Paciottib, Christine Wu Nordahlb, c, Sally Ozonoffb, c, Judy A. Van de Waterb, d, Sally J. Rogersb, c, David Amaralb, c, Paul Ashwood

a Department of Medical Microbiology and Immunology, University of California, Davis, USA
b The M.I.N.D. Institute, University of California, Davis, USA
c Department of Psychiatry and Behavioral Sciences, University of California, Davis, USA
d Division of Rheumatology, Allergy and Clinical Immunology, University of California, Davis, USA

Abstract
The pathophysiology of autism spectrum disorder (ASD) is not yet known; however, studies suggest that dysfunction of the immune system affects many children with ASD. Increasing evidence points to dysfunction of the innate immune system including activation of microglia and perivascular macrophages, increases in inflammatory cytokines/chemokines in brain tissue and CSF, and abnormal peripheral monocyte cell function. Dendritic cells are major players in innate immunity and have important functions in the phagocytosis of pathogens or debris, antigen presentation, activation of naïve T cells, induction of tolerance and cytokine/chemokine production. In this study, we assessed circulating frequencies of myeloid dendritic cells (defined as Lin-1−BDCA1+CD11c+ and Lin-1−BDCA3+CD123−) and plasmacytoid dendritic cells (Lin-1−BDCA2+CD123+ or Lin-1−BDCA4+ CD11c−) in 57 children with ASD, and 29 typically developing controls of the same age, all of who were enrolled as part of the Autism Phenome Project (APP). The frequencies of dendritic cells and associations with behavioral assessment and MRI measurements of amygdala volume were compared in the same participants. The frequencies of myeloid dendritic cells were significantly increased in children with ASD compared to typically developing controls ($p < 0.03$). Elevated frequencies of myeloid dendritic cells were positively associated with abnormal right and left amygdala enlargement, severity of gastrointestinal symptoms and increased repetitive behaviors. The frequencies of plasmacytoid dendritic cells were also associated with amygdala volumes as well as developmental regression in children with ASD. **Dendritic cells play key roles in modulating immune responses and differences in frequencies or functions of these cells may result in immune dysfunction in children with ASD. These data further implicate innate immune cells in the complex pathophysiology of ASD.**

33. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosal

    Environmental Health Perspectives, Aug 2005.

    Thomas Burbacher, PhD [University of Washington].

    This study demonstrates clearly and unequivocally that ethyl mercury, the kind of mercury found in vaccines, not only ends up in the brain, but leaves double the amount of inorganic mercury as methyl mercury, the kind of mercury found in fish. Methyl mercury (organic mercury) has a half-life in the brain measured in

days (Rice), while thimerosal (organic mercury) once in the brain converts to inorganic mercury at much higher rates, and inorganic mercury has a half-life in the brain measured in years and decades (Rooney). This work is groundbreaking because little is known about ethyl mercury, and many health authorities have asserted that the mercury found in vaccines is the "safe kind." This study also delivers a strong rebuke of the Institute of Medicine's recommendation in 2004 to no longer pursue the mercury-autism connection.

Excerpt: "A recently published IOM review (IOM 2004) appears to have abandoned the earlier recommendation [of studying mercury and autism] as well as back away from the American Academy of Pediatrics goal [of removing mercury from vaccines]. This approach is difficult to understand, given our current limited knowledge of the toxicokinetics and developmental neurotoxicity of thimerosal, a compound that has been (and will continue to be) injected in millions of newborns and infants."

Excerpt: “ The average brain-to-blood partitioning ratio of total Hg in the thimerosal group was slightly higher than that in the MeHg group (3.5 ± 0.5 vs. 2.5 ± 0.3, t-test, p = 0.11). **Thus, the brain to-blood Hg concentration ratio established for MeHg will underestimate the amount of Hg in the brain after exposure to thimerosal.** “

Abstract
Thimerosal is a preservative that has been used in manufacturing vaccines since the 1930s. Reports have indicated that infants can receive ethylmercury (in the form of thimerosal) at or above the U.S. Environmental Protection Agency guidelines for methylmercury exposure, depending on the exact vaccinations, schedule, and size of the infant. In this study we compared the systemic disposition and brain distribution of total and inorganic mercury in infant monkeys after thimerosal exposure with those exposed to MeHg. Monkeys were exposed to MeHg (via oral gavage) or vaccines containing thimerosal (via intramuscular injection) at birth and 1, 2, and 3 weeks of age. Total blood Hg levels were determined 2, 4, and 7 days after each exposure. Total and inorganic brain Hg levels were assessed 2, 4, 7, or 28 days after the last exposure. The initial and terminal half-life of Hg in blood after thimerosal exposure was 2.1 and 8.6 days, respectively, which are significantly shorter than the elimination half-life of Hg after MeHg exposure at 21.5 days. Brain concentrations of total Hg were significantly lower by approximately 3-fold for the thimerosal-exposed monkeys when compared with the MeHg infants, whereas the average brain-to-blood concentration ratio was slightly higher for the thimerosal-exposed monkeys (3.5 ± 0.5 vs. 2.5 ± 0.3). **A higher percentage of the total Hg in the brain was in the form of inorganic Hg for the thimerosal-exposed monkeys (34% vs. 7%).** The results indicate that MeHg is not a suitable reference for risk assessment from exposure to thimerosal-derived Hg. Knowledge of the toxicokinetics and developmental toxicity of thimerosal is needed to afford a meaningful assessment of the developmental effects of thimerosal-containing vaccines. Key words: brain and blood distribution, elimination half-life, ethylmercury, infant nonhuman primates, methylmercury, thimerosal.

34. The retention time of inorganic mercury in the brain--a systematic review of the evidence.

Toxicol Appl Pharmacol. 2014 Feb 1;274(3):425-35. doi: 10.1016/j.taap.2013.12.011. Epub 2013 Dec 22.

Rooney JP.

Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College, 152-160 Pearse Street, Dublin 2, Ireland. Electronic address: jrooney@rcsi.ie.

Abstract
Reports from human case studies indicate a half-life for inorganic mercury in the brain in the order of years-contradicting older radioisotope studies that estimated half-lives in the order of weeks to months in duration. This study systematically reviews available evidence on the retention time of inorganic mercury in humans and primates to better understand this conflicting evidence. A broad search strategy was used to capture 16,539 abstracts on the Pubmed database. Abstracts were screened to include only study types containing relevant information. 131 studies of interest were identified. Only 1 primate study made a numeric estimate for the half-life of inorganic mercury (227-540 days). Eighteen human mercury poisoning cases were followed up long term including autopsy. Brain inorganic mercury concentrations at death were consistent with a half-life of several years or longer. 5 radionucleotide studies were found, one of which estimated head half-life (21 days). This estimate has sometimes been misinterpreted to be equivalent to brain half-life-which ignores several confounding factors including limited radioactive half-life and radioactive decay from surrounding tissues including circulating blood. No autopsy cohort study estimated a half-life for inorganic mercury, although some noted bioaccumulation of brain mercury with age. Modelling studies provided some extreme estimates (69 days vs 22 years). Estimates from modelling studies appear sensitive to model assumptions, however predications based on a long half-life (27.4 years) are consistent with autopsy findings. In summary, shorter estimates of half-life are not supported by evidence from animal studies, human case studies, or modelling studies based on appropriate assumptions. **Evidence from such studies point to a half-life of inorganic mercury in human brains of several years to several decades.** This finding carries important implications for pharmcokinetic modelling of mercury and potentially for the regulatory toxicology of mercury.

35. Brain and tissue levels of mercury after chronic methylmercury exposure in the monkey.

J Toxicol Environ Health. 1989;27(2):189-98.

Rice DC

Toxicology Research Division, Health Protection Branch, Health and Welfare, Ottawa, Ontario, Canada.

Abstract
Estimated half-lives of mercury following methylmercury exposure in humans are 52-93 d for whole body and 49-164 d for blood. In its most recent 1980 review, the World Health Organization concluded that there was no evidence to suggest that brain half-life differed from whole-body half-life. In the present study, female monkeys (Macaca fascicularis) were dosed for at least 1.7 yr with 10, 25, or 50 micrograms/kg.d of mercury as methylmercuric chloride. Dosing was discontinued, and blood half-life was determined to be about 14 d. Approximately 230 d after cessation of dosing, monkeys were sacrificed and organ and regional brain total mercury levels determined. One monkey that died while still being dosed had brain mercury levels three times higher than levels in blood. Theoretical calculations were performed assuming steady-state brain:blood ratios of 3, 5, or 10. Brain mercury levels were at least three orders of magnitude higher than those predicted by assuming the half-life in brain to be the same as that in blood. Estimated half-lives in brain were between 56 (brain:blood ratio of 3) and 38 (brain:blood ratio of 10) days. In addition, there was a dose-dependent difference in half-lives for some brain regions. These data clearly indicate that brain half-life is considerably longer than blood half-life in the monkey under conditions of chronic dosing.

36. Increases in the number of reactive glia in the visual cortex of Macaca fascicularis following subclinical long-term methyl mercury exposure.

Toxicology and Applied Pharmacology, 1994

Charleston JS, Bolender RP, Mottet NK, Body RL, Vahter ME, Burbacher TM., Department of Pathology, School of Medicine, University of Washington

Abstract
The number of neurons, astrocytes, reactive glia, oligodendrocytes, endothelia, and pericytes in the cortex of the calcarine sulcus of adult female Macaca fascicularis following long-term subclinical exposure to methyl mercury (MeHg) and mercuric chloride (inorganic mercury; IHg) has been estimated by use of the optical volume fractionator stereology technique. Four groups of monkeys were exposed to MeHg (50 micrograms Hg/kg body wt/day) by mouth for 6, 12, 18, and 12 months followed by 6 months without exposure (clearance group). A fifth group of monkeys was administered IHg (as HgCl2; 200 micrograms Hg/kg body wt/day) by constant rate intravenous infusion via an indwelling catheter for 3 months. Reactive glia showed a significant increase in number for every treatment group, increasing 72% in the 6-month, 152% in the 12-month, and 120% in the 18-month MeHg exposed groups, and the number of reactive glia in the clearance group remained elevated (89%). The IHg exposed group showed a 165% increase in the number of reactive glia. The IHg exposed group and the clearance group had low levels of MeHg present within the tissue; however, the level of IHg was elevated in both groups. **These results suggest that the IHg**

may be responsible for the increase in reactive glia. All other cell types, including the neurons, showed no significant change in number at the prescribed exposure level and durations. The identities of the reactive glial cells and the implications for the long-term function and survivability of the neurons due to changes in the glial population following subclinical long-term exposure to mercury are discussed.

37. Neuroglial Activation and Neuroinflammation in the Brain of Patients with Autism

    Annals of Neurology, Feb 2005.

    Diana L. Vargas, MD, Johns Hopkins University.

    Abstract
    Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. To investigate whether immune-mediated mechanisms are involved in the pathogenesis of autism, we used immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent assays to study brain tissues and cerebrospinal fluid (CSF) from autistic patients and determined the magnitude of neuroglial and inflammatory reactions and their cytokine expression profiles. Brain tissues from cerebellum, midfrontal, and cingulate gyrus obtained at autopsy from 11 patients with autism were used for morphological studies. Fresh-frozen tissues available from seven patients and CSF from six living autistic patients were used for cytokine protein profiling. We demonstrate an active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemoattractant protein (MCP)-1 and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1. Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain.

    Excerpt: "**Because this neuroinflammatory process appears to be associated with an ongoing and chronic mechanism of CNS dysfunction, potential therapeutic interventions should focus on the control of its detrimental effects and thereby eventually modify the clinical course of autism**."

38. Transcriptome analysis reveals dysregulation of innate immune response genes and neuronal activity-dependent genes in autism

Nature Communications 5, Article number: 5748 doi:10.1038/ncomms6748
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Department of Medicine, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA
Simone Gupta, Shannon E. Ellis, Foram N. Ashar, Anna Moes, Joel S. Bader
Dan E. Arking
Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA
Joel S. Bader & Jianan Zhan
Department of Neurology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA
Andrew B. West

Abstract
Recent studies of genomic variation associated with autism have suggested the existence of extreme heterogeneity. Large-scale transcriptomics should complement these results to identify core molecular pathways underlying autism. Here we report results from a large-scale RNA sequencing effort, utilizing region-matched autism and control brains to identify neuronal and microglial genes robustly dysregulated in autism cortical brain. **Remarkably, we note that a gene expression module corresponding to M2-activation states in microglia is negatively correlated with a differentially expressed neuronal module, implicating dysregulated microglial responses in concert with altered neuronal activity-dependent genes in autism brains.** These observations provide pathways and candidate genes that highlight the interplay between innate immunity and neuronal activity in the aetiology of autism.

39. Nanomolar aluminum induces pro-inflammatory and pro-apoptotic gene expression in human brain cells in primary culture.

J Inorg Biochem. 2005 Sep;99(9):1895-8.

Lukiw WJ1, Percy ME, Kruck TP.

Neuroscience Center of Excellence and Department of Ophthalmology, Louisiana State University Health Sciences Center, 2020 Gravier Street, Suite 8B8, New Orleans, LA 70112-2272, USA. wlukiw@lsuhsc.edu

Abstract
Aluminum, the most abundant neurotoxic metal in our biosphere, has been implicated in the etiology of several neurodegenerative disorders including Alzheimer's disease (AD). To further understand aluminum's influence on gene expression, we examined total messenger RNA levels in untransformed human neural cells exposed to 100 nanomolar aluminum sulfate using high density DNA microarrays that interrogate the expression of every human gene. Preliminary data indicate that of the most altered gene expression levels, 17/24 (70.8%) of

aluminum-affected genes, and 7/8 (87.5%) of aluminum-induced genes exhibit expression patterns similar to those observed in AD. The seven genes found to be significantly up-regulated by aluminum encode pro-inflammatory or pro-apoptotic signaling elements, including NF-kappaB subunits, interleukin-1beta precursor, cytosolic phospholipase A2, cyclooxygenase-2, beta-amyloid precursor protein and DAXX, a regulatory protein known to induce apoptosis and repress transcription. **The promoters of genes up-regulated by aluminum are enriched in binding sites for the stress-inducible transcription factors HIF-1 and NF-kappaB, suggesting a role for aluminum, HIF-1 and NF-kappaB in driving atypical, pro-inflammatory and pro-apoptotic gene expression.** The effect of aluminum on specific stress-related gene expression patterns in human brain cells clearly warrant further investigation.

40. Aberrant NF-kappaB expression in autism spectrum condition: a mechanism for neuroinflammation.

Front Psychiatry. 2011 May 13;2:27. doi: 10.3389/fpsyt.2011.00027. eCollection 2011.

Young AM1, Campbell E, Lynch S, Suckling J, Powis SJ.

Bute Medical School, University of St. Andrews Fife, Scotland, UK.

Abstract
Autism spectrum condition (ASC) is recognized as having an inflammatory component. Post-mortem brain samples from patients with ASC display neuroglial activation and inflammatory markers in cerebrospinal fluid, although little is known about the underlying molecular mechanisms. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein found in almost all cell types and mediates regulation of immune response by inducing the expression of inflammatory cytokines and chemokines, establishing a feedback mechanism that can produce chronic or excessive inflammation. This article describes immunodetection and immunofluorescence measurements of NF-κB in human post-mortem samples of orbitofrontal cortex tissue donated to two independent centers: London Brain Bank, Kings College London, UK (ASC: n = 3, controls: n = 4) and Autism Tissue Program, Harvard Brain Bank, USA (ASC: n = 6, controls: n = 5). The hypothesis was that concentrations of NF-κB would be elevated, especially in activated microglia in ASC, and pH would be concomitantly reduced (i.e., acidification). Neurons, astrocytes, and microglia all demonstrated increased extranuclear and nuclear translocated NF-κB p65 expression in brain tissue from ASC donors relative to samples from matched controls. These between-groups differences were increased in astrocytes and microglia relative to neurons, but particularly pronounced for highly mature microglia. Measurement of pH in homogenized samples demonstrated a 0.98-unit difference in means and a strong ($F = 98.3$; $p = 0.00018$) linear relationship to the expression of nuclear translocated NF-κB in mature microglia. Acridine orange staining localized pH reductions to lysosomal compartments. **In summary, NF-κB is aberrantly expressed in orbitofrontal cortex in patients with ASC, as part of a putative molecular cascade leading to inflammation,**

especially of resident immune cells in brain regions associated with the behavioral and clinical symptoms of ASC.

41. A Study of Nuclear Transcription Factor-Kappa B in Childhood Autism

PLoS One. 2011; 6(5): e19488.

Usha S. Naik,1 Charitha Gangadharan,2 Kanakalatha Abbagani,1 Balakrishna Nagalla,3 Niranjan Dasari,1 and Sunil K. Manna2,*
Monica Uddin, Editor

Department of Psychiatry, Osmania Medical College, Hyderabad, India
Laboratory of Immunology, Centre for DNA Fingerprinting and Diagnostics, Nampally, Hyderabad, India
National Institute of Nutrition, Hyderabad, India
University of Michigan, United States of America

Abstract
Background
Several children with autism show regression in language and social development while maintaining normal motor milestones. A clear period of normal development followed by regression and subsequent improvement with treatment, suggests a multifactorial etiology. The role of inflammation in autism is now a major area of study. Viral and bacterial infections, hypoxia, or medication could affect both foetus and infant. These stressors could upregulate transcription factors like nuclear factor kappa B (NF-κB), a master switch for many genes including some implicated in autism like tumor necrosis factor (TNF). On this hypothesis, it was proposed to determine NF-κB in children with autism.

Methods
Peripheral blood samples of 67 children with autism and 29 control children were evaluated for NF-κB using electrophoretic mobility shift assay (EMSA). A phosphor imaging technique was used to quantify values. The fold increase over the control sample was calculated and statistical analysis was carried out using SPSS 15.

Results
We have noted significant increase in NF-κB DNA binding activity in peripheral blood samples of children with autism. When the fold increase of NF-κB in cases (n=67) was compared with that of controls (n=29), there was a significant difference (3.14 vs. 1.40, respectively; p<0.02).

Conclusion
This finding has immense value in understanding many of the known biochemical changes reported in autism. As NF-κB is a response to stressors of several kinds and a master switch for many genes, autism may then arise at least in part from an NF-κB pathway gone awry.

42. Autism: A Brain Disorder, or A Disorder That Affects the Brain?

Clinical Neuropsychiatry, 2005

Martha R. Herbert M.D., Ph.D., Harvard University

Autism is defined behaviorally, as a syndrome of abnormalities involving language, social reciprocity and hyperfocus or reduced behavioral flexibility. It is clearly heterogeneous, and it can be accompanied by unusual talents as well as by impairments, but its underlying biological and genetic basis in unknown. Autism has been modeled as a brain-based, strongly genetic disorder, but emerging findings and hypotheses support a broader model of the condition as a genetically influenced and systemic. These include imaging, neuropathology and psychological evidence of pervasive (and not just specific) brain and phenotypic features; postnatal evolution and chronic persistence of brain, behavior and tissue changes (e.g. inflammation) and physical illness symptomatology (e.g. gastrointestinal, immune, recurrent infection); overlap with other disorders; and reports of rate increases and improvement or recovery that support a role for modulation of the condition by environmental factors (e.g. exacerbation or triggering by toxins, infectious agents, or others stressors, or improvement by treatment). Modeling autism more broadly encompasses previous work, but also encourages the expansion of research and treatment to include intermediary domains of molecular and cellular mechanisms, as well as chronic tissue, metabolic and somatic changes previously addressed only to a limited degree. The heterogeneous biologies underlying autism may conceivably converge onto the autism profile via multiple mechanisms on the one hand and processing and connectivity abnormalities on the other may illuminate relevant final common pathways and contribute to focusing on the search for treatment targets in this biologically and etiologically heterogeneous behavioral syndrome.

43. Activation of Methionine Synthase by Insulin-like Growth Factor-1 and Dopamine: a Target for Neurodevelopmental Toxins and Thimerosal

Mol Psychiatry. 2004 Apr;9(4):358-70.

Waly M, Olteanu H, Banerjee R, Choi SW, Mason JB, Parker BS, Sukumar S, Shim S, Sharma A, Benzecry JM, Power-Charnitsky VA, Deth RC. Department of Pharmaceutical Sciences, Northeastern University, Boston, MA

Abstract
Methylation events play a critical role in the ability of growth factors to promote normal development. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might

exert adverse effects on methylation. We found that insulin-like growth factor-1 (IGF-1)- and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells, via a PI3-kinase- and MAP-kinase-dependent mechanism. The stimulation of this pathway increased DNA methylation, while its inhibition increased methylation-sensitive gene expression. Ethanol potently interfered with IGF-1 activation of MS and blocked its effect on DNA methylation, whereas it did not inhibit the effects of dopamine. Metal ions potently affected IGF-1 and dopamine-stimulated MS activity, as well as folate-dependent phospholipid methylation: Cu(2+) promoted enzyme activity and methylation, while Cu(+), Pb(2+), Hg(2+) and Al(3+) were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC(50) of 1 nM and eliminated MS activity. Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. **The potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins**.

44. Validation of the Phenomenon of Autistic Regression Using Home Videotapes

Archives of General Psychiatry, 2005

Emily Werner, PhD; Geraldine Dawson, PhD, University of Washington

Abstract
Objective To validate parental report of autistic regression using behavioral data coded from home videotapes of children with autism spectrum disorder (ASD) vs typical development taken at 12 and 24 months of age.

Design Home videotapes of 56 children’s first and second birthday parties were collected from parents of young children with ASD with and without a reported history of regression and typically developing children. Child behaviors were coded by raters blind to child diagnosis and regression history. A parent interview that elicited information about parents’ recall of early symptoms from birth was also administered.

Setting Participants were recruited from a multidisciplinary study of autism conducted at a major university.

Participants Fifteen children with ASD with a history of regression, 21 children with ASD with early-onset autism, and 20 typically developing children and their parents participated.

Main Outcome Measures Observations of children’s communicative, social, affective, repetitive behaviors, and toy play coded from videotapes of the toddlers’ first and second birthday parties.

Results Analyses revealed that infants with ASD with regression show similar use of joint attention and more frequent use of words and babble compared with

typical infants at 12 months of age. In contrast, infants with ASD with early onset of symptoms and no regression displayed fewer joint attention and communicative behaviors at 12 months of age. By 24 months of age, both groups of toddlers with ASD displayed fewer instances of word use, vocalizations, declarative pointing, social gaze, and orienting to name as compared with typically developing 24-month-olds.

Parent interview data suggested that some children with regression displayed difficulties in regulatory behavior before the regression occurred.

Conclusion **This study validates the existence of early autistic regression**.

45. Blood Levels of Mercury Are Related to Diagnosis of Autism: A Reanalysis of an Important Data Set

Journal of Child Neurology, Vol. 22, No. 11, 1308-1311 (2007)

M. Catherine DeSoto, PhD, Robert T. Hitlan, PhD -Department of Psychology, University of Northern Iowa, Cedar Falls, Iowa

Abstract
The question of what is leading to the apparent increase in autism is of great importance. Like the link between aspirin and heart attack, even a small effect can have major health implications. If there is any link between autism and mercury, it is absolutely crucial that the first reports of the question are not falsely stating that no link occurs. **We have reanalyzed the data set originally reported by Ip et al. in 2004 and have found that the original p value was in error and that a significant relation does exist between the blood levels of mercury and diagnosis of an autism spectrum disorder. Moreover, the hair sample analysis results offer some support for the idea that persons with autism may be less efficient and more variable at eliminating mercury from the blood.**

46. Empirical Data Confirm Autism Symptoms Related to Aluminum and Acetaminophen Exposure

Entropy, November 7, 2012

Stephanie Seneff, Robert M. Davidson and Jingjing Liu

Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA, Internal Medicine Group Practice, PhyNet, Inc., Longview, TX 75604, USA

Abstract

Autism is a condition characterized by impaired cognitive and social skills, associated with compromised immune function. The incidence is alarmingly on the rise, and environmental factors are increasingly suspected to play a role. This paper investigates word frequency patterns in the U.S. CDC Vaccine Adverse Events Reporting System (VAERS) database. Our results provide strong evidence supporting a link between autism and the aluminum in vaccines. A literature review showing toxicity of aluminum in human physiology offers further support. Mentions of autism in VAERS increased steadily at the end of the last century, during a period when mercury was being phased out, while aluminum adjuvant burden was being increased. Using standard log-likelihood ratio techniques, we identify several signs and symptoms that are significantly more prevalent in vaccine reports after 2000, including cellulitis, seizure, depression, fatigue, pain and death, which are also significantly associated with aluminum-containing vaccines. **We propose that children with the autism diagnosis are especially vulnerable to toxic metals such as aluminum and mercury due to insufficient serum sulfate and glutathione. A strong correlation between autism and the MMR (Measles, Mumps, Rubella) vaccine is also observed, which may be partially explained via an increased sensitivity to acetaminophen administered to control fever.**

47. Glutathione-related factors and oxidative stress in autism, a review.

Curr Med Chem. 2012;19(23):4000-5.

Ghanizadeh A1, Akhondzadeh S, Hormozi M, Makarem A, Abotorabi-Zarchi M, Firoozabadi A.

Research Center for Psychiatry and Behavioral Sciences, Shiraz University of Medical Sciences, School of Medicine, Shiraz, Iran. ghanizad@sina.tums.ac.ir

Abstract
Autism spectrum disorders are complex neuro-developmental disorders whose neurobiology is proposed to be associated with oxidative stress which is induced by reactive oxygen species. The process of oxidative stress can be a target for therapeutic interventions. In this study, we aimed to review the role of oxidative stress, plasma glutathione (GSH), and related factors as the potential sources of damage to the brain as well as the possible related factors which reduce the oxidative stress. Methylation capacity, sulfates level, and the total glutathione level are decreased in autism. On the other hand, both oxidized glutathione and the ratio of oxidized to reduced glutathione are increased in autism. In addition, the activity of glutathione peroxidase, superoxide dismutase, and catalase, as a part of the antioxidative stress system are decreased. **The current literature suggests an imbalance of oxidative and anti-oxidative stress systems in autism. Glutathione is involved in neuro-protection against oxidative stress and neuro-inflammation in autism by improving the anti-oxidative stress system. Decreasing the oxidative stress might be a potential treatment for autism.**

48. Developmental Regression and Mitochondrial Dysfunction in a Child With Autism

J Child Neurol. 2006 Feb;21(2):170-2.

Jon S. Poling, MD, PhD, Department of Neurology and Neurosurgery
Johns Hopkins Hospital

Abstract
Autistic spectrum disorders can be associated with mitochondrial dysfunction. We present a singleton case of developmental regression and oxidative phosphorylation disorder in a 19-month-old girl. Subtle abnormalities in the serum creatine kinase level, aspartate aminotransferase, and serum bicarbonate led us to perform a muscle biopsy, which showed type I myofiber atrophy, increased lipid content, and reduced cytochrome c oxidase activity. There were marked reductions in enzymatic activities for complex I and III. Complex IV (cytochrome c oxidase) activity was near the 5% confidence level. To determine the frequency of routine laboratory abnormalities in similar patients, we performed a retrospective study including 159 patients with autism (Diagnostic and Statistical Manual of Mental Disorders-IV and Childhood Autism Rating Scale) not previously diagnosed with metabolic disorders and 94 age-matched controls with other neurologic disorders. Aspartate aminotransferase was elevated in 38% of patients with autism compared with 15% of controls ($P < .0001$). The serum creatine kinase level also was abnormally elevated in 22 (47%) of 47 patients with autism. These data suggest that further metabolic evaluation is indicated in autistic patients and that defects of oxidative phosphorylation might be prevalent.

Excerpt: "**Children who have (mitochondrial-related) dysfunctional cellular energy metabolism might be more prone to undergo autistic regression between 18 and 30 months of age if they also have infections or immunizations at the same time**."

49. Oxidative Stress in Autism: Elevated Cerebellar 3-nitrotyrosine Levels

American Journal of Biochemistry and Biotechnology 4 (2): 73-84, 2008

Elizabeth M. Sajdel-Sulkowska, - Dept of Psychiatry, Harvard Medical School

Shows a potential link between mercury and the autopsied brains of young people with autism. A marker for oxidative stress was 68.9% higher in autistic brain issue than controls (a statistically significant result), while mercury levels were 68.2% higher.

Abstract
It has been suggested that oxidative stress and/or mercury compounds play an

important role in the pathophysiology of autism. This study compared for the first time the cerebellar levels of the oxidative stress marker 3-nitrotyrosine (3-NT), mercury (Hg) and the antioxidant selenium (Se) levels between control and autistic subjects. Tissue homogenates were prepared in the presence of protease inhibitors from the frozen cerebellar tissue of control (n=10; mean age, 15.5 years; mean PMI, 15.5 hours) and autistic (n=9; mean age 12.1 years; mean PMI, 19.3 hours) subjects. The concentration of cerebellar 3-NT, determined by ELISA, in controls ranged from 13.69 to 49.04 pmol g^{-1} of tissue; the concentration of 3-NT in autistic cases ranged from 3.91 to 333.03 pmol g^{-1} of tissue. Mean cerebellar 3-NT was elevated in autism by 68.9% and the increase was statistically significant (p=0.045). Cerebellar Hg, measured by atomic absorption spectrometry ranged from 0.9 to 35 pmol g^{-1} tissue in controls (n=10) and from 3.2 to 80.7 pmol g^{-1} tissue in autistic cases (n=9); the 68.2% increase in cerebellar Hg was not statistically significant. However, there was a positive correlation between cerebellar 3-NT and Hg levels (r=0.7961, p=0.0001). A small decrease in cerebellar Se levels in autism, measured by atomic absorption spectroscopy, was not statistically significant but was accompanied by a 42.9% reduction in the molar ratio of Se to Hg in the autistic cerebellum. While preliminary, the results of the present study add elevated oxidative stress markers in brain to the growing body of data reflecting greater oxidative stress in autism.

Excerpt: **The preliminary data suggest a need for more extensive studies of oxidative stress, its relationship to the environmental factors and its possible attenuation by antioxidants in autism**."

50. Large Brains in Autism: The Challenge of Pervasive Abnormality

Neuroscientist. 2005 Oct;11(5):417-40.

Herbert MR., Harvard University

Pediatric Neurology, Center for Morphometric Analysis, Massachusetts General Hospital, Charleston, MA

Abstract
The most replicated finding in autism neuroanatomy-a tendency to unusually large brains-has seemed paradoxical in relation to the specificity of the abnormalities in three behavioral domains that define autism. We now know a range of things about this phenomenon, including that brains in autism have a growth spurt shortly after birth and then slow in growth a few short years afterward, that only younger but not older brains are larger in autism than in controls, that white matter contributes disproportionately to this volume increase and in a nonuniform pattern suggesting postnatal pathology, that functional connectivity among regions of autistic brains is diminished, and that neuroinflammation (including microgliosis and astrogliosis) appears to be present in autistic brain tissue from childhood through adulthood. Alongside these pervasive brain tissue and functional abnormalities, there have arisen theories of pervasive or widespread neural information processing or signal coordination

abnormalities (such as weak central coherence, impaired complex processing, and underconnectivity), which are argued to underlie the specific observable behavioral features of autism. This convergence of findings and models suggests that a systems- and chronic disease-based reformulation of function and pathophysiology in autism needs to be considered, and it opens the possibility for new treatment targets..

Excerpt: "**Oxidative stress, brain inflammation, and microgliosis have been much documented in association with toxic exposures including various heavy metals...the awareness that the brain as well as medical conditions of children with autism may be conditioned by chronic biomedical abnormalities such as inflammation opens the possibility that meaningful biomedical interventions may be possible well past the window of maximal neuroplasticity in early childhood because the basis for assuming that all deficits can be attributed to fixed early developmental alterations in neural architecture has now been undermined**."

51. Evidence of Toxicity, Oxidative Stress, and Neuronal Insult in Autism

J Toxicol Environ Health B Crit Rev. 2006 Nov-Dec;9(6):485-99.

Kern JK, Jones AM.

Department of Psychiatry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas

Abstract
According to the Autism Society of America, autism is now considered to be an epidemic. The increase in the rate of autism revealed by epidemiological studies and government reports implicates the importance of external or environmental factors that may be changing. This article discusses the evidence for the case that some children with autism may become autistic from neuronal cell death or brain damage sometime after birth as result of insult; and addresses the hypotheses that toxicity and oxidative stress may be a cause of neuronal insult in autism. The article first describes the Purkinje cell loss found in autism, Purkinje cell physiology and vulnerability, and the evidence for postnatal cell loss. Second, the article describes the increased brain volume in autism and how it may be related to the Purkinje cell loss. Third, the evidence for toxicity and oxidative stress is covered and the possible involvement of glutathione is discussed. Finally, the article discusses what may be happening over the course of development and the multiple factors that may interplay and make these children more vulnerable to toxicity, oxidative stress, and neuronal insult.

52. Oxidative Stress in Autism

Pathophysiology. 2006 Aug;13(3):171-81. Epub 2006 Jun 12.

Chauhan A, Chauhan V.

NYS Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY

Abstract
Autism is a severe developmental disorder with poorly understood etiology. Oxidative stress in autism has been studied at the membrane level and also by measuring products of lipid peroxidation, detoxifying agents (such as glutathione), and antioxidants involved in the defense system against reactive oxygen species (ROS). Lipid peroxidation markers are elevated in autism, indicating that oxidative stress is increased in this disease. Levels of major antioxidant serum proteins, namely transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein), are decreased in children with autism. There is a positive correlation between reduced levels of these proteins and loss of previously acquired language skills in children with autism. The alterations in ceruloplasmin and transferrin levels may lead to abnormal iron and copper metabolism in autism. The membrane phospholipids, the prime target of ROS, are also altered in autism. The levels of phosphatidylethanolamine (PE) are decreased, and phosphatidylserine (PS) levels are increased in the erythrocyte membrane of children with autism as compared to their unaffected siblings. Several studies have suggested alterations in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in autism. Additionally, altered glutathione levels and homocysteine/methionine metabolism, increased inflammation, excitotoxicity, as well as mitochondrial and immune dysfunction have been suggested in autism. Furthermore, environmental and genetic factors may increase vulnerability to oxidative stress in autism. Taken together, these studies suggest increased oxidative stress in autism that may contribute to the development of this disease. A mechanism linking oxidative stress with membrane lipid abnormalities, inflammation, aberrant immune response, impaired energy metabolism and excitotoxicity, leading to clinical symptoms and pathogenesis of autism is proposed.

Excerpt: "**Upon completion of this article, participants should be able to: 1. Be aware of laboratory and clinical evidence of greater oxidative stress in autism. 2. Understand how gut, brain, nutritional, and toxic status in autism are consistent with greater oxidative stress. 3. Describe how anti-oxidant nutrients are used in the contemporary treatment of autism.**"

53. Thimerosal Neurotoxicity is Associated with Glutathione Depletion: Protection with Glutathione Precursors

Neurotoxicology. 2005 Jan;26(1):1-8.

James SJ, Slikker W 3rd, Melnyk S, New E, Pogribna M, Jernigan S.

Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR

Abstract
Thimerosol is an antiseptic containing 49.5% ethyl mercury that has been used for years as a preservative in many infant vaccines and in flu vaccines. Environmental methyl mercury has been shown to be highly neurotoxic, especially to the developing brain. Because mercury has a high affinity for thiol (sulfhydryl (-SH)) groups, the thiol-containing antioxidant, glutathione (GSH), provides the major intracellular defense against mercury-induced neurotoxicity. Cultured neuroblastoma cells were found to have lower levels of GSH and increased sensitivity to thimerosol toxicity compared to glioblastoma cells that have higher basal levels of intracellular GSH. **Thimerosal-induced cytotoxicity was associated with depletion of intracellular GSH in both cell lines.** Pretreatment with 100 microM glutathione ethyl ester or N-acetylcysteine (NAC), but not methionine, resulted in a significant increase in intracellular GSH in both cell types. Further, pretreatment of the cells with glutathione ethyl ester or NAC prevented cytotoxicity with exposure to 15 microM Thimerosal. Although Thimerosal has been recently removed from most children's vaccines, it is still present in flu vaccines given to pregnant women, the elderly, and to children in developing countries. The potential protective effect of GSH or NAC against mercury toxicity warrants further research as possible adjunct therapy to individuals still receiving Thimerosal-containing vaccinations.

54. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage.

Curr Top Med Chem. 2001 Dec;1(6):529-39.

Ercal N1, Gurer-Orhan H, Aykin-Burns N.

University of Missouri-Rolla, Department of Chemistry, 65409-0010, USA. nercal@umr.edu

Abstract
Toxic metals (lead, cadmium, mercury and arsenic) are widely found in our environment. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Recent studies indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities associated with these metals might be due to oxidative tissue damage. Redox-active metals, such as iron, copper and chromium, undergo redox cycling whereas redox-inactive metals, such as lead, cadmium, mercury and others deplete cells' major antioxidants, particularly thiol-containing antioxidants and enzymes. Either redox-active or redox-inactive metals may cause an increase in production of reactive oxygen species (ROS) such as hydroxyl radical (HO.), superoxide radical (O2.-) or hydrogen peroxide (H2O2). Enhanced generation of ROS can overwhelm cells' intrinsic antioxidant defenses, and result in a condition known as "oxidative stress". Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA. **Consequently, it is suggested that metal-induced**

oxidative stress in cells can be partially responsible for the toxic effects of heavy metals. Several studies are underway to determine the effect of antioxidant supplementation following heavy metal exposure. Data suggest that antioxidants may play an important role in abating some hazards of heavy metals. In order to prove the importance of using antioxidants in heavy metal poisoning, pertinent biochemical mechanisms for metal-induced oxidative stress should be reviewed.

55. Aluminum adjuvant linked to gulf war illness induces motor neuron death in mice

Neuromolecular Med. 2007;9(1):83-100.

Petrik MS, Wong MC, Tabata RC, Garry RF, Shaw CA.

Department of Ophthalmology and Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada.

Abstract
Gulf War illness (GWI) affects a significant percentage of veterans of the 1991 conflict, but its origin remains unknown. Associated with some cases of GWI are increased incidences of amyotrophic lateral sclerosis and other neurological disorders. Whereas many environmental factors have been linked to GWI, the role of the anthrax vaccine has come under increasing scrutiny. Among the vaccine's potentially toxic components are the adjuvants aluminum hydroxide and squalene. To examine whether these compounds might contribute to neuronal deficits associated with GWI, an animal model for examining the potential neurological impact of aluminum hydroxide, squalene, or aluminum hydroxide combined with squalene was developed. Young, male colony CD-1 mice were injected with the adjuvants at doses equivalent to those given to US military service personnel. All mice were subjected to a battery of motor and cognitive-behavioral tests over a 6-mo period postinjections. Following sacrifice, central nervous system tissues were examined using immunohistochemistry for evidence of inflammation and cell death. Behavioral testing showed motor deficits in the aluminum treatment group that expressed as a progressive decrease in strength measured by the wire-mesh hang test (final deficit at 24 wk; about 50%). Significant cognitive deficits in water-maze learning were observed in the combined aluminum and squalene group (4.3 errors per trial) compared with the controls (0.2 errors per trial) after 20 wk. Apoptotic neurons were identified in aluminum-injected animals that showed significantly increased activated caspase-3 labeling in lumbar spinal cord (255%) and primary motor cortex (192%) compared with the controls. Aluminum-treated groups also showed significant motor neuron loss (35%) and increased numbers of astrocytes (350%) in the lumbar spinal cord. The findings suggest a possible role for the aluminum adjuvant in some neurological features associated with GWI and possibly an additional role for the combination of adjuvants.

56. Enrichment of Elevated Plasma F2t-Isoprostane Levels in Individuals with Autism Who Are Stratified by Presence of Gastrointestinal Dysfunction

PLoS ONE 8(7): e68444.

Gorrindo P, Lane CJ, Lee EB, McLaughlin B, Levitt P (July 3, 2013)

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AbstractEtiology is unknown in the majority of individuals with autism spectrum disorder (ASD). One strategy to investigate pathogenesis is to stratify this heterogeneous disorder based on a prominent phenotypic feature that enriches for homogeneity within population strata. Co-occurring gastrointestinal dysfunction (GID) characterizes a subset of children with ASD. Our current objective was to investigate a potential pathophysiological measure to test the hypothesis that children with both ASD and GID have a more severe metabolic dysfunction than children with ASD-only, given that the highly metabolically active brain and gastrointestinal system may additively contribute measurable impairment. Plasma levels of F2t-Isoprostanes (F2-IsoPs), a gold standard biomarker of oxidative stress, were measured in 87 children in four groups: ASD-GID, ASD-only, GID-only and Unaffected. F2-IsoP levels were elevated in all 3 clinical groups compared to the Unaffected group, with the ASD-GID group significantly elevated above the ASD-only group (mean, SD in pg/mg: ASD-GID 53.6, 24.4; ASD-only 36.5, 13.3; p = 0.007). Adjusting for age, sex, and triglyceride levels, F2-IsoP levels remained significantly different between study groups, with a moderate effect size of ηp2 = 0.187 (p = 0.001). **Elevation in peripheral oxidative stress is consistent with, and may contribute to, the more severe functional impairments in the ASD-GID group.** With unique medical, metabolic, and behavioral features in children with ASD-GID, the present findings serve as a compelling rationale for both individualized approaches to clinical care and integrated studies of biomarker enrichment in ASD subgroups that may better address the complex etiology of ASD.

57. Reduced levels of mercury in first baby haircuts of autistic children.

Int J Toxicol. 2003 Jul-Aug;22(4):277-85.

Holmes AS, Blaxill MF, Haley BE.

Abstract
Reported rates of autism have increased sharply in the United States and the United Kingdom. One possible factor underlying these increases is increased exposure to mercury through thimerosal-containing vaccines, but vaccine exposures need to be evaluated in the context of cumulative exposures during gestation and early infancy. Differential rates of postnatal mercury elimination may explain why similar gestational and infant exposures produce variable neurological effects. First baby haircut samples were obtained from 94 children diagnosed with autism using Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM IV) criteria and 45 age- and gender-matched controls. Information on diet, dental amalgam fillings, vaccine history, Rho D immunoglobulin administration, and autism symptom severity was collected through a maternal survey questionnaire and clinical observation. Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively. Hair mercury levels among controls were significantly correlated with the number of the mothers' amalgam fillings and their fish consumption as well as exposure to mercury through childhood vaccines, correlations that were absent in the autistic group. Hair excretion patterns among autistic infants were significantly reduced relative to control. These data cast doubt on the efficacy of traditional hair analysis as a measure of total mercury exposure in a subset of the population. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, the present study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.

58. A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorder

J Toxicol Environ Health A. 2007 May 15;70(10):837-51.

Geier DA, Geier MR.

Institute of Chronic Illnesses, Inc., Silver Spring, Maryland, USA.

Abstract

Impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns characterize autism spectrum disorders (ASDs). It is clear that while genetic factors are important to the pathogenesis of ASDs, mercury exposure can induce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASDs. The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, U.S. Department of Health and Human Services, IRB number IRB00005375) approved the present study. A case series of nine patients

who presented to the Genetic Centers of America for a genetic/developmental evaluation are discussed. Eight of nine patients (one patient was found to have an ASD due to Rett's syndrome) (a) had regressive ASDs; (b) had elevated levels of androgens; (c) excreted significant amounts of mercury post chelation challenge; (d) had biochemical evidence of decreased function in their glutathione pathways; (e) had no known significant mercury exposure except from Thimerosal-containing vaccines/Rho(D)-immune globulin preparations; and (f) had alternate causes for their regressive ASDs ruled out. There was a significant dose-response relationship between the severity of the regressive ASDs observed and the total mercury dose children received from Thimerosal-containing vaccines/Rho (D)-immune globulin preparations. Based upon differential diagnoses, 8 of 9 patients examined were exposed to significant mercury from Thimerosal-containing biologic/vaccine preparations during their fetal/infant developmental periods, and subsequently, between 12 and 24 mo of age, **these previously normally developing children suffered mercury toxic encephalopathies that manifested with clinical symptoms consistent with regressive ASDs. Evidence for mercury intoxication should be considered in the differential diagnosis as contributing to some regressive ASDs**.

59. The Changing Prevalence of Autism In California

Journal of Autism and Developmental Disorders, April 2003
Mark Blaxill, MBA

This study helps to refute the supposition made by some researchers that autism's epidemic may only be due to "diagnostic substitution".

Excerpt: "**They have suggested that 'diagnostic substitution' accounts for an apparent increase in the incidence of autism in California that is not real. This hypothesized substitution is not supported by proper and detailed analyses of the California data**."

60. Mitochondrial Energy-Deficient Endophenotype in Autism

American Journal of Biochemistry and Biotechnology 4 (2): 198-207, 2008

J. Jay Gargus and Faiqa Imtiaz
Department of Physiology and Biophysics and Department of Pediatrics, Section of Human Genetics, School of Medicine, University of California, Irvine, Arabian Diagnostics Laboratory, King Faisal Specialist Hospital and Research Centre

Abstract: While evidence points to a multigenic etiology of most autism, the pathophysiology of the disorder has yet to be defined and the underlying genes and biochemical pathways they subserve remain unknown. Autism is considered to be influenced by a combination of various genetic, environmental and immunological factors; more recently, evidence has suggested that increased

vulnerability to oxidative stress may be involved in the etiology of this multifactorial disorder.

Furthermore, recent studies have pointed to a subset of autism associated with the biochemical endophenotype of mitochondrial energy deficiency, identified as a subtle impairment in fat and carbohydrate oxidation. This phenotype is similar, but more subtle than those seen in classic mitochondrial defects. In some cases the beginnings of the genetic underpinnings of these mitochondrial defects are emerging, such as mild mitochondrial dysfunction and secondary carnitine deficiency observed in the subset of autistic patients with an inverted duplication of chromosome 15q11-q13. In addition, rare cases of familial autism associated with sudden infant death syndrome (SIDS) or associated with abnormalities in cellular calcium homeostasis, such as malignant hyperthermia or cardiac arrhythmia, are beginning to emerge. **Such special cases suggest that the pathophysiology of autism may comprise pathways that are directly or indirectly involved in mitochondrial energy production** and to further probe this connection three new avenues seem worthy of exploration: 1) metabolomic clinical studies provoking controlled aerobic exercise stress to expand the biochemical phenotype, 2) high-throughput expression arrays to directly survey activity of the genes underlying these biochemical pathways and 3) model systems, either based upon neuronal stem cells or model genetic organisms, to discover novel genetic and environmental inputs into these pathways.

61. Bridging from Cells to Cognition in Autism Pathophysiology: Biological Pathways to Defective Brain Function and Plasticity

American Journal of Biochemistry and Biotechnology 4 (2): 167-176, 2008

Matthew P. Anderson, Brian S. Hooker and Martha R. Herbert
Departments of Neurology and Pathology, Harvard Medical School/Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, High Throughput Biology Team, Fundamental Science Directorate, Pacific Northwest National Laboratory, Pediatric Neurology/Center for Morphometric Analysis, Massachusetts General Hospital/Harvard Medical School, and Center for Child and Adolescent Development, Cambridge Health Alliance/Harvard Medical School

Abstract: We review evidence to support a model where the disease process underlying autism may begin when an in utero or early postnatal environmental, infectious, seizure, or autoimmune insult triggers an immune response that increases reactive oxygen species (ROS) production in the brain that leads to DNA damage (nuclear and mitochondrial) and metabolic enzyme blockade and that these inflammatory and oxidative stressors persist beyond early development (with potential further exacerbations), producing ongoing functional consequences. In organs with a high metabolic demand such as the central nervous system, the continued use of mitochondria with damaged DNA and impaired metabolic enzyme function may generate additional ROS which will

cause persistent activation of the innate immune system leading to more ROS production. Such a mechanism would self-sustain and possibly progressively worsen. The mitochondrial dysfunction and altered redox signal transduction pathways found in autism would conspire to activate both astroglia and microglia. These activated cells can then initiate a broad-spectrum proinflammatory gene response. Beyond the direct effects of ROS on neuronal function, receptors on neurons that bind the inflammatory mediators may serve to inhibit neuronal signaling to protect them from excitotoxic damage during various pathologic insults (e.g., infection). **In autism, over-zealous neuroinflammatory responses could not only influence neural developmental processes, but may more significantly impair neural signaling involved in cognition in an ongoing fashion. T**his model makes specific predictions in patients and experimental animal models and suggests a number of targets sites of intervention. Our model of potentially reversible pathophysiological mechanisms in autism motivates our hope that effective therapies may soon appear on the horizon.

62. Heavy-Metal Toxicity—With Emphasis on Mercury

John Neustadt, ND, and Steve Pieczenik, MD, PhD

Research Review

Conclusion: Metals are ubiquitous in our environment, and exposure to them is inevitable. However, not all people accumulate toxic levels of metals or exhibit symptoms of metal toxicity, suggesting that genetics play a role in their potential to damage health. **Metal toxicity creates multisystem dysfunction, which appears to be mediated primarily through mitochondrial damage from glutathione depletion.**
Accurate screening can increase the likelihood that patients with potential metal toxicity are identified. The most accurate screening method for assessing chronic-metals exposure and metals load in the body is a provoked urine test.

63. Evidence of Mitochondrial Dysfunction in Autism and Implications for Treatment

American Journal of Biochemistry and Biotechnology 4 (2): 208-217, 2008

Daniel A. Rossignol, J. Jeffrey Bradstreet, International Child Development Resource Center,

Abstract
Classical mitochondrial diseases occur in a subset of individuals with autism and are usually caused by genetic anomalies or mitochondrial respiratory pathway deficits. However, in many cases of autism, there is evidence of mitochondrial dysfunction (MtD) without the classic features associated with mitochondrial

disease. MtD appears to be more common in autism and presents with less severe signs and symptoms. It is not associated with discernable mitochondrial pathology in muscle biopsy specimens despite objective evidence of lowered mitochondrial functioning. **Exposure to environmental toxins is the likely etiology for MtD in autism. This dysfunction then contributes to a number of diagnostic symptoms and comorbidities observed in autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. MtD and oxidative stress may also explain the high male to female ratio found in autism due to increased male vulnerability to these dysfunctions.**

Biomarkers for mitochondrial dysfunction have been identified, but seem widely under-utilized despite available therapeutic interventions. Nutritional supplementation to decrease oxidative stress along with factors to improve reduced glutathione, as well as hyperbaric oxygen therapy (HBOT) represent supported and rationale approaches. The underlying pathophysiology and autistic symptoms of affected individuals would be expected to either improve or cease worsening once effective treatment for MtD is implemented.

64. Proximity to point sources of environmental mercury release as a predictor of autism prevalence

    Health & Place, 2008

    Raymond F. Palmer, Stephen Blanchard, Robert Wood
    University of Texas Health Science Center, San Antonio Department of Family and Community Medicine, Our Lady of the Lake University, San Antonio Texas, Chair, Department of Sociology

    This study should be viewed as hypothesis-generating - a first step in examining the potential role of environmental mercury and childhood developmental disorders. Nothing is known about specific exposure routes, dosage, timing, and individual susceptibility. **We suspect that persistent low-dose exposures to various environmental toxicants, including mercury, that occur during critical windows of neural development among genetically susceptible children (with a diminished capacity for metabolizing accumulated toxicants) may increase the risk for developmental disorders such as autism.** Successfully identifying the specific combination of environmental exposures and genetic susceptibilities can inform the development of targeted prevention intervention strategies.

65. Epidemiology of autism spectrum disorder in Portugal: prevalence, clinical characterization, and medical conditions

Developmental Medicine & Child Neurology, 2007

Guiomar Oliveira MD PhD, Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra; Assunção Ataíde BSc, Direcção Regional de Educação do Centro Coimbra;
Carla Marques MSc, Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra; Teresa S Miguel BSc, Direcção Regional de Educação do Centro, Coimbra;
Ana Margarida Coutinho BSc, Instituto Gulbenkian de Ciência, Oeiras; Luísa Mota-Vieira PhD, Unidade de Genética e Patologia moleculares, Hospital do Divino Espírito Santo, Ponta Delgada, Açores; Esmeralda Gonçalves PhD; Nazaré Mendes Lopes PhD, Faculdade de Ciências e Tecnologia, Universidade de Coimbra; Vitor Rodrigues MD PhD; Henrique Carmona da Mota MD PhD, Faculdade de Medicina, Universidade de Coimbra, Coimbra; Astrid Moura Vicente PhD, Instituto Gulbenkian de Ciência, Oeiras, Portugal.
*Correspondence to first author at Hospital Pediátrico de Coimbra, Av Bissaya Barreto, 3000-076 Coimbra, Portugal. E-mail: guiomar@hpc.chc.min-saude.pt

Abstract: The objective of this study was to estimate the prevalence of autistic spectrum disorder (ASD) and identify its clinical characterization, and medical conditions in a paediatric population in Portugal. A school survey was conducted in elementary schools, targeting 332 808 school-aged children in the mainland and 10 910 in the Azores islands. Referred children were directly assessed using the Diagnostic and Statistical Manual of Mental Disorders (4th edn), the Autism Diagnostic Interview–Revised, and the Childhood Autism Rating Scale. Clinical history and a laboratory investigation was performed. In parallel, a systematic multi-source search of children known to have autism was carried out in a restricted region. The global prevalence of ASD per 10 000 was 9.2 in mainland, and 15.6 in the Azores, with intriguing regional differences. **A diversity of associated medical conditions was documented in 20%, with an unexpectedly high rate of mitochondrial respiratory chain disorders.**

66. Thimerosal induces neuronal cell apoptosis by causing cytochrome c and apoptosis-inducing factor release from mitochondria.

International Journal of Molecular Medicine, 2006

Yel L, Brown LE, Su K, Gollapudi S, Gupta S.Department of Medicine, University of California, Irvine, CA 92697, USA. lyel@uci.edu

There is a worldwide increasing concern over the neurological risks of thimerosal (ethylmercury thiosalicylate) which is an organic mercury compound that is commonly used as an antimicrobial preservative. In this study, we show that thimerosal, at nanomolar concentrations, induces neuronal cell death through the mitochondrial pathway. Thimerosal, in a concentration- and time-dependent manner, decreased cell viability as assessed by calcein-ethidium staining and

caused apoptosis detected by Hoechst 33258 dye. Thimerosal-induced apoptosis was associated with depolarization of mitochondrial membrane, generation of reactive oxygen species, and release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria to cytosol. Although thimerosal did not affect cellular expression of Bax at the protein level, we observed translocation of Bax from cytosol to mitochondria. Finally, caspase-9 and caspase-3 were activated in the absence of caspase-8 activation. Our data suggest that thimerosal causes apoptosis in neuroblastoma cells by changing the mitochondrial microenvironment.

67. Mitochondrial mediated thimerosal-induced apoptosis in a human neuroblastoma cell line (SK-N-SH).

Neurotoxicology. 2005

Humphrey ML, Cole MP, Pendergrass JC, Kiningham KK. Department of Pharmacology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25704-9388, USA.

Environmental exposure to mercurials continues to be a public health issue due to their deleterious effects on immune, renal and neurological function. Recently the safety of thimerosal, an ethyl mercury-containing preservative used in vaccines, has been questioned due to exposure of infants during immunization. Mercurials have been reported to cause apoptosis in cultured neurons; however, the signaling pathways resulting in cell death have not been well characterized. Therefore, the objective of this study was to identify the mode of cell death in an in vitro model of thimerosal-induced neurotoxicity, and more specifically, to elucidate signaling pathways which might serve as pharmacological targets. Within 2 h of thimerosal exposure (5 microM) to the human neuroblastoma cell line, SK-N-SH, morphological changes, including membrane alterations and cell shrinkage, were observed. Cell viability, assessed by measurement of lactate dehydrogenase (LDH) activity in the medium, as well as the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, showed a time- and concentration-dependent decrease in cell survival upon thimerosal exposure. In cells treated for 24 h with thimerosal, fluorescence microscopy indicated cells undergoing both apoptosis and oncosis/necrosis. To identify the apoptotic pathway associated with thimerosal-mediated cell death, we first evaluated the mitochondrial cascade, as both inorganic and organic mercurials have been reported to accumulate in the organelle. Cytochrome c was shown to leak from the mitochondria, followed by caspase 9 cleavage within 8 h of treatment. In addition, poly(ADP-ribose) polymerase (PARP) was cleaved to form a 85 kDa fragment following maximal caspase 3 activation at 24 h. Taken together these findings suggest deleterious effects on the cytoarchitecture by thimerosal and initiation of mitochondrial-mediated apoptosis.

68. Possible Immunological Disorders in Autism: Concomitant Autoimmunity and Immune Tolerance

The Egyptian Journal of Immunology, 2006

Maha I. Sh. Kawashti, Omnia R. Amin Nadia G. Rowehy

Microbiology Department, Faculty of Medicine (For Girls), Al Azhar University, Cairo, Egypt, Psychiatry Department, Faculty of Medicine, Cairo University, Cairo, Egypt and Serology Lab King Fahad General Hospital, Jeddah, K.S.A.

Abstract: Autism is a pervasive developmental disorder that affect children early in their life. Immunological disorders is one of several contributing factors that have been suggested to cause autism. Thirty autistic children aged 3-6 years and thirty non-autistic psychologically-free siblings were studied. Circulating IgA and IgG autoantibodies to casein and gluten dietary proteins were detected by enzyme-immunoassays (EIA). Circulating IgG antibodies to measles, mumps and rubella vaccine (M.M.R) and cytomeglovirus were investigated by EIA. Results revealed high seropositivity for autoantibodies to casein and gluten: 83.3% and 50% respectively in autistic children as compared to 10% and 6.7% positivity in the control group. Surprisingly, circulating anti-measles, anti-mumps and anti-rubella IgG were positive in only 50%, 73.3% and 53.3% respectively as compared to 100% positivity in the control group. Anti-CMV IgG was positive in 43.3% of the autistic children as compared to 7% in the control group. It is concluded that, autoimmune response to dietary proteins and deficient immune response to measles, mumps and rubella vaccine antigens might be associated with autism, as a leading cause or a resulting event. Further research is needed to confirm these findings.

69. [Pediatric Vaccines Influence Primate Behavior, and Amygdala Growth and Opioid Ligand Binding]

Friday, May 16, 2008: IMFAR

L. Hewitson , Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA B. Lopresti , Radiology, University of Pittsburgh, Pittsburgh, PA C. Stott , Thoughtful House Center for Children, Austin, TX J. Tomko , Pittsburgh Development Center, University of Pittsburgh, Pittsburgh, PA L. Houser , Pittsburgh Development Center, University of Pittsburgh, Pittsburgh, PA E. Klein , Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, PA C. Castro , Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA G. Sackett , Psychology, Washington National Primate Research Center, Seattle, WA S. Gupta , Medicine, Pathology & Laboratory Medicine, University of California - Irvine, Irvine, CA D. Atwood , Chemistry, University of Kentucky, Lexington, KY L. Blue , Chemistry, University

of Kentucky, Lexington, KY E. R. White , Chemistry, University of Kentucky, Lexington, KY A. Wakefield , Thoughtful House Center for Children, Austin, TX

Abstract
Background: Macaques are commonly used in pre-clinical vaccine safety testing, but the combined childhood vaccine regimen, rather than individual vaccines, has not been studied. Childhood vaccines are a possible causal factor in autism, and abnormal behaviors and anomalous amygdala growth are potentially inter-related features of this condition.

Objectives: The objective of this study was to compare early infant cognition and behavior with amygdala size and opioid binding in rhesus macaques receiving the recommended childhood vaccines (1994-1999), the majority of which contained the bactericidal preservative ethylmercurithiosalicylic acid (thimerosal).

Methods: Macaques were administered the recommended infant vaccines, adjusted for age and thimerosal dose (exposed; N=13), or saline (unexposed; N=3). Primate development, cognition and social behavior were assessed for both vaccinated and unvaccinated infants using standardized tests developed at the Washington National Primate Research Center. Amygdala growth and binding were measured serially by MRI and by the binding of the non-selective opioid antagonist [11C]diprenorphine, measured by PET, respectively, before (T1) and after (T2) the administration of the measles-mumps-rubella vaccine (MMR).

Results: **Compared with unexposed animals, significant neurodevelopmental deficits were evident for exposed animals in survival reflexes, tests of color discrimination and reversal, and learning sets. Differences in behaviors were observed between exposed and unexposed animals and within the exposed group before and after MMR vaccination. Compared with unexposed animals, exposed animals showed attenuation of amygdala growth and differences in the amygdala binding of [11C]diprenorphine. Interaction models identified significant associations between specific aberrant social and non-social behaviors, isotope binding, and vaccine exposure.**

Conclusions: **This animal model, which examines for the first time, behavioral, functional, and neuromorphometric consequences of the childhood vaccine regimen, mimics certain neurological abnormalities of autism. The findings raise important safety issues** while providing a potential model for examining aspects of causation and disease pathogenesis in acquired disorders of behavior and development.

70. Thimerosal exposure in infants and neurodevelopmental disorders: An assessment of computerized medical records in the Vaccine Safety Datalink.

Young HA, Geier DA, Geier MR.

The George Washington University School of Public Health and Health Services, Department of Epidemiology and Biostatistics, United States.

Abstract
The study evaluated possible associations between neurodevelopmental disorders (NDs) and exposure to mercury (Hg) from Thimerosal-containing vaccines (TCVs) by examining the automated Vaccine Safety Datalink (VSD). A total of 278,624 subjects were identified in birth cohorts from 1990-1996 that had received their first oral polio vaccination by 3 months of age in the VSD. The birth cohort prevalence rate of medically diagnosed International Classification of Disease, 9th revision (ICD-9) specific NDs and control outcomes were calculated. Exposures to Hg from TCVs were calculated by birth cohort for specific exposure windows from birth-7 months and birth-13 months of age. Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from TCVs. **Consistent significantly increased rate ratios were observed for autism, autism spectrum disorders, tics, attention deficit disorder, and emotional disturbances with Hg exposure from TCVs.** By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs. Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be conducted to further evaluate the relationship between Hg exposure and NDs.

71. Glutathione, oxidative stress and neurodegeneration

Schulz JB, Lindenau J, Seyfried J, Dichgans J.
Neurodegeneration Laboratory, Department of Neurology, University of Tübingen, Germany.

Eur J Biochem. 2000 Aug;267(16):4904-11.

Abstract
There is significant evidence that the pathogenesis of several neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, Friedreich's ataxia and amyotrophic lateral sclerosis, may involve the generation of reactive oxygen species and mitochondrial dysfunction. Here, we review the evidence for a disturbance of glutathione homeostasis that may either lead to or result from oxidative stress in neurodegenerative disorders. Glutathione is an important intracellular antioxidant that protects against a variety of different antioxidant species. An important role for glutathione was proposed for the pathogenesis of Parkinson's disease, because a decrease in total glutathione concentrations in the substantia nigra has been observed in preclinical stages, at a time at which other biochemical changes are not yet detectable. Because glutathione does not cross the blood-brain barrier other treatment options to increase brain concentrations of glutathione including glutathione analogs, mimetics or precursors are discussed.

72. Hepatitis B triple series vaccine and developmental disability in US children aged 1-9 years

Carolyn Gallagher a; Melody Goodman, Graduate Program in Public Health, Stony Brook University Medical Center, Health Sciences Center, New York, USA

Journal Toxicological & Environmental Chemistry, Volume 90, Issue 5 September 2008 , pages 997 - 1008

Abstract
This study investigated the association between vaccination with the Hepatitis B triple series vaccine prior to 2000 and developmental disability in children aged 1–9 years (n = 1824), proxied by parental report that their child receives early intervention or special education services (EIS). National Health and Nutrition Examination Survey 1999–2000 data were analyzed and adjusted for survey design by Taylor Linearization using SAS version 9.1 software, with SAS callable SUDAAN version 9.0.1. The odds of receiving EIS were approximately nine times as great for vaccinated boys (n = 46) as for unvaccinated boys (n = 7), after adjustment for confounders. **This study found statistically significant evidence to suggest that boys in United States who were vaccinated with the triple series Hepatitis B vaccine, during the time period in which vaccines were manufactured with thimerosal, were more susceptible to developmental disability than were unvaccinated boys**.

73. Induction of metallothionein in mouse cerebellum and cerebrum with low-dose thimerosal injection.

Minami T, Miyata E, Sakamoto Y, Yamazaki H, Ichida S., Department of Life Sciences, School of Science & Engineering, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka, 577-8502, Japan, minamita@life.kindai.ac.jp.

Cell Biology and Toxicology. 2009 Apr 9. [Epub ahead of print]

Abstract
Thimerosal, an ethyl mercury compound, is used worldwide as a vaccine preservative. We previously observed that the mercury concentration in mouse brains did not increase with the clinical dose of thimerosal injection, but the concentration increased in the brain after the injection of thimerosal with lipopolysaccharide, even if a low dose of thimerosal was administered. Thimerosal may penetrate the brain, but is undetectable when a clinical dose of thimerosal is injected; therefore, the induction of metallothionein (MT) messenger RNA (mRNA) and protein was observed in the cerebellum and cerebrum of mice after thimerosal injection, as MT is an inducible protein. MT-1 mRNA was expressed at 6 and 9 h in both the cerebrum and cerebellum, but MT-1 mRNA

expression in the cerebellum was three times higher than that in the cerebrum after the injection of 12 microg/kg thimerosal. MT-2 mRNA was not expressed until 24 h in both organs. MT-3 mRNA was expressed in the cerebellum from 6 to 15 h after the injection, but not in the cerebrum until 24 h. MT-1 and MT-3 mRNAs were expressed in the cerebellum in a dose-dependent manner. Furthermore, MT-1 protein was detected from 6 to 72 h in the cerebellum after 12 microg/kg of thimerosal was injected and peaked at 10 h. MT-2 was detected in the cerebellum only at 10 h. In the cerebrum, little MT-1 protein was detected at 10 and 24 h, and there were no peaks of MT-2 protein in the cerebrum. In conclusion, MT-1 and MT-3 mRNAs but not MT-2 mRNA are easily expressed in the cerebellum rather than in the cerebrum by the injection of low-dose thimerosal. It is thought that the cerebellum is a sensitive organ against thimerosal. **As a result of the present findings, in combination with the brain pathology observed in patients diagnosed with autism, the present study helps to support the possible biological plausibility for how low-dose exposure to mercury from thimerosal-containing vaccines may be associated with autism.**

74. Mercury induces inflammatory mediator release from human mast cells

Duraisamy Kempuraj, Shahrzad Asadi, Bodi Zhang, Akrivi Manola, Jennifer Hogan, Erika Peterson, Theoharis C Theoharides

Journal of Neuroinflammation 2010, 7:20 doi:10.1186/1742-2094-7-20

Abstract
Background: Mercury is known to be neurotoxic, but its effects on the immune system are less well known. Mast cells are involved in allergic reactions, but also in innate and acquired immunity, as well as in inflammation. Many patients with Autism Spectrum Disorders (ASD) have “allergic” symptoms; moreover, the prevalence of ASD in patients with mastocytosis, characterized by numerous hyperactive mast cells in most tissues, is 10-fold higher than the general population suggesting mast cell involvement. We, therefore, investigated the effect of mercuric chloride (HgCl2) on human mast cell activation.

Methods: Human leukemic cultured LAD2 mast cells and normal human umbilical cord bloodderived cultured mast cells (hCBMCs) were stimulated by HgCl2 (0.1-10 μM) for either 10 min for beta-hexosaminidase release or 24 hr for measuring vascular endothelial growth factor (VEGF) and IL-6 release by ELISA.

Results: HgCl2 induced a 2-fold increase in β-hexosaminidase release, and also significant VEGF release at 0.1 and 1 μM (311±32 pg/106 cells and 443±143 pg/106 cells, respectively) from LAD2 mast cells compared to control cells (227±17 pg/106 cells, n=5, p<0.05). Addition of HgCl2 (0.1 μM) to the proinflammatory neuropeptide substance P (SP, 0.1 μM) had synergestic action in inducing VEGF from LAD2 mast cells. HgCl2 also stimulated significant VEGF release (360 ± 100 pg/106 cells at 1 μM, n=5, p<0.05) from hCBMCs compared to control cells (182 ±57 pg/106 cells), and IL-6 release (466±57 pg/106 cells at

0.1 µM) compared to untreated cells (13±25 pg/106 cells, n=5, $p<0.05$). Addition of HgCl2 (0.1 µM) to SP (5 µM) further increased IL-6 release. Conclusions: HgCl2 stimulates VEGF and IL-6 release from human mast cells. **This phenomenon could disrupt the blood-brain-barrier and permit brain inflammation. As a result, the findings of the present study provide a biological mechanism for how low levels of mercury may contribute to ASD pathogenesis.**

75. Influence of pediatric vaccines on amygdala growth and opioid ligand binding in rhesus macaque infants: A pilot study

Acta Neurobiol Exp 2010, 70: 147–164 Polish Neuroscience Society - PTBUN, Nencki Institute of Experimental Biology

Laura Hewitson1,2,*, Brian J. Lopresti3, Carol Stott4, N. Scott Mason3 and Jaime Tomko1

Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; Thoughtful House Center for Children, Austin, TX, USA; Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; 4Independent Chartered Scientist, Cambridge, UK;

Abstract
This longitudinal, case-control pilot study examined amygdala growth in rhesus macaque infants receiving the complete US childhood vaccine schedule (1994-1999). Longitudinal structural and functional neuroimaging was undertaken to examine central effects of the vaccine regimen on the developing brain. Vaccine-exposed and saline-injected control infants underwent MRI and PET imaging at approximately 4 and 6 months of age, representing two specific timeframes within the vaccination schedule. Volumetric analyses showed that exposed animals did not undergo the maturational changes over time in amygdala volume that was observed in unexposed animals. After controlling for left amygdala volume, the binding of the opioid antagonist [11C]diprenorphine (DPN) in exposed animals remained relatively constant over time, compared with unexposed animals, in which a significant decrease in [11C]DPN binding occurred. **These results suggest that maturational changes in amygdala volume and the binding capacity of [11C]DPN in the amygdala was significantly altered in infant macaques receiving the vaccine schedule. The macaque infant is a relevant animal model in which to investigate specific environmental exposures and structural/functional neuroimaging during neurodevelopment.**

76. Cultured lymphocytes from autistic children and non-autistic siblings up-regulate heat shock protein RNA in response to thimerosal challenge.

Neurotoxicology. 2006 Sep;27(5):685-92. Epub 2006 Jun 16.

Walker SJ, Segal J, Aschner M.

Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27156, USA. swalker@wfubmc.edu
Abstract

Abstract
There are reports suggesting that some autistic children are unable to mount an adequate response following exposure to environmental toxins. This potential deficit, coupled with the similarity in clinical presentations of autism and some heavy metal toxicities, has led to the suggestion that heavy metal poisoning might play a role in the etiology of autism in uniquely susceptible individuals. Thimerosal, an anti-microbial preservative previously added routinely to childhood multi-dose vaccines, is composed of 49.6% ethyl mercury. Based on the levels of this toxin that children receive through routine immunization schedules in the first years of life, it has been postulated that thimerosal may be a potential triggering mechanism contributing to autism in susceptible individuals. One potential risk factor in these individuals may be an inability to adequately up-regulate metallothionein (MT) biosynthesis in response to presentation of a heavy metal challenge. To investigate this hypothesis, cultured lymphocytes (obtained from the Autism Genetic Resource Exchange, AGRE) from autistic children and non-autistic siblings were challenged with either 10 microM ethyl mercury, 150 microM zinc, or fresh media (control). Following the challenge, total RNA was extracted and used to query "whole genome" DNA microarrays. Cultured lymphocytes challenged with zinc responded with an impressive up-regulation of MT transcripts (at least nine different MTs were over-expressed) **while cells challenged with thimerosal responded by up-regulating numerous heat shock protein transcripts, but not MTs. Although there were no apparent differences between autistic and non-autistic sibling responses in this very small sampling group, the differences in expression profiles between those cells treated with zinc versus thimerosal were dramatic.** Determining cellular response, at the level of gene expression, has important implications for the understanding and treatment of conditions that result from exposure to neurotoxic compounds.

77. Sorting out the spinning of autism: heavy metals and the question of incidence

Acta Neurobiol Exp 2010, 70: 165–176

Mary Catherine DeSoto* and Robert T. Hitlan, Department of Psychology, University of Northern Iowa, Cedar Falls, Iowa, USA

The reasons for the rise in autism prevalence are a subject of heated professional debate. Featuring a critical appraisal of some research used to

question whether there is a rise in cases and if rising levels of autism are related to environmental
exposure to toxins (Soden et al. 2007, Thompson et al. 2007, Barbaresi et al. 2009) we aim to evaluate the actual state of scientific knowledge. In addition, we surveyed the empirical research on the topic of autism and heavy metal toxins. Overall, the various causes that have led to the increase in autism diagnosis are likely multi-faceted, and understanding the causes is one of the most important health topics today. We argue that scientific research does not support rejecting the link between the neurodevelopmental disorder of autism and toxic exposures.

78. Urinary Porphyrin Excretion in Neurotypical and Autistic Children

Environ Health Perspect. 2010 Oct;118(10):1450-7. Epub 2010 Jun 24.

Woods JS, Armel SE, Fulton DI, Allen J, Wessels K, Simmonds PL, Granpeesheh D, Mumper E, Bradstreet JJ, Echeverria D, Heyer NJ, Rooney JP., Department of Environmental and Occupational Health Sciences, University of Washington

Abstract
BACKGROUND: Increased urinary concentrations of pentacarboxyl-, precopro- and copro-porphyrins have been associated with prolonged mercury (Hg) exposure in adults, and comparable increases have been attributed to Hg exposure in children with autism (AU).

OBJECTIVES: This study was designed to measure and compare urinary porphyrin concentrations in neurotypical (NT) children and same-age children with autism, and to examine the association between porphyrin levels and past or current Hg exposure in children with autism.

METHODS: This exploratory study enrolled 278 children 2-12 years of age. We evaluated three groups: AU, pervasive developmental disorder-not otherwise specified (PDD-NOS), and NT. Mothers/caregivers provided information at enrollment regarding medical, dental, and dietary exposures. Urine samples from all children were acquired for analyses of porphyrin, creatinine, and Hg. Differences between groups for mean porphyrin and Hg levels were evaluated. Logistic regression analysis was conducted to determine whether porphyrin levels were associated with increased risk of autism.

RESULTS: Mean urinary porphyrin concentrations are naturally high in young children and decline by as much as 2.5-fold between 2 and 12 years of age. Elevated copro- ($p < 0.009$), hexacarboxyl- ($p < 0.01$) and pentacarboxyl- ($p < 0.001$) porphyrin concentrations were significantly associated with AU but not with PDD-NOS. No differences were found between NT and AU in urinary Hg levels or in past Hg exposure as determined by fish consumption, number of dental amalgam fillings, or vaccines received. CONCLUSIONS:**These findings identify disordered porphyrin metabolism as a salient characteristic of**

autism. Hg exposures were comparable between diagnostic groups, and a porphyrin pattern consistent with that seen in Hg-exposed adults was not apparent.

79. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

Molecular Psychiatry advance online publication 25 January 2011;doi: 10.1038/mp.2010.136

D A Rossignol and R E Frye

Abstract
A comprehensive literature search was performed to collate evidence of mitochondrial dysfunction in autism spectrum disorders (ASDs) with two primary objectives. First, features of mitochondrial dysfunction in the general population of children with ASD were identified. Second, characteristics of mitochondrial dysfunction in children with ASD and concomitant mitochondrial disease (MD) were compared with published literature of two general populations: ASD children without MD, and non-ASD children with MD. The prevalence of MD in the general population of ASD was 5.0% (95% confidence interval 3.2, 6.9%), much higher than found in the general population (~0.01%). The prevalence of abnormal biomarker values of mitochondrial dysfunction was high in ASD, much higher than the prevalence of MD. Variances and mean values of many mitochondrial biomarkers (lactate, pyruvate, carnitine and ubiquinone) were significantly different between ASD and controls. Some markers correlated with ASD severity. Neuroimaging, in vitro and post-mortem brain studies were consistent with an elevated prevalence of mitochondrial dysfunction in ASD. Taken together, these findings suggest children with ASD have a spectrum of mitochondrial dysfunction of differing severity. Eighteen publications representing a total of 112 children with ASD and MD (ASD/MD) were identified. The prevalence of developmental regression (52%), seizures (41%), motor delay (51%), gastrointestinal abnormalities (74%), female gender (39%), and elevated lactate (78%) and pyruvate (45%) was significantly higher in ASD/MD compared with the general ASD population. The prevalence of many of these abnormalities was similar to the general population of children with MD, suggesting that ASD/MD represents a distinct subgroup of children with MD. Most ASD/MD cases (79%) were not associated with genetic abnormalities, raising the possibility of secondary mitochondrial dysfunction. Treatment studies for ASD/MD were limited, although improvements were noted in some studies with carnitine, co-enzyme Q10 and B-vitamins. Many studies suffered from limitations, including small sample sizes, referral or publication biases, and variability in protocols for selecting children for MD workup, collecting mitochondrial biomarkers and defining MD. **Overall, this evidence supports the notion that mitochondrial dysfunction is associated with ASD.** Additional studies are needed to further define the role of mitochondrial dysfunction in ASD.

80. Sensitization effect of thimerosal is mediated in vitro via reactive oxygen species and calcium signaling.

Toxicology. 2010 July - August;274(1-3):1-9. Epub 2010 May 10.

Migdal C, Foggia L, Tailhardat M, Courtellemont P, Haftek M, Serres M.

Thimerosal, a mercury derivative composed of ethyl mercury chloride (EtHgCl) and thiosalicylic acid (TSA), is widely used as a preservative in vaccines and cosmetic products and causes cutaneous reactions. Since dendritic cells (DCs) play an essential role in the immune response, the sensitization potency of chemicals was studied in vitro using U937, a human promyelomonocytic cell line that is used as a surrogate of monocytic differentiation and activation. Currently, this cell line is under ECVAM (European Center for the Validation of Alternative Methods) validation as an alternative method for discriminating chemicals. Thimerosal and mercury derivatives induced in U937 an overexpression of CD86 and interleukin (IL)-8 secretion similarly to 1-chloro-2,4-dinitrobenzene (DNCB), a sensitizer used as a positive control for DC activation. Non-sensitizers, dichloronitrobenzene (DCNB), TSA and sodium dodecyl sulfate (SDS), an irritant, had no effect. U937 activation was prevented by cell pretreatment with N-acetyl-l-cysteine (NAC) but not with thiol-independent antioxidants except vitamin E which affected CD86 expression by preventing lipid peroxidation of cell membranes. Thimerosal, EtHgCl and DNCB induced glutathione (GSH) depletion and reactive oxygen species (ROS) within 15min; another peak was detected after 2h for mercury compounds only. MitoSOX, a specific mitochondrial fluorescent probe, confirmed that ROS were essentially produced by mitochondria in correlation with its membrane depolarization. Changes in mitochondrial membrane permeability induced by mercury were reversed by NAC but not by thiol-independent antioxidants. Thimerosal and EtHgCl also induced a calcium (Ca(2+)) influx with a peak at 3h, suggesting that Ca(2+) influx is a secondary event following ROS induction as Ca(2+) influx was suppressed after pretreatment with NAC but not with thiol-independent antioxidants. Ca(2+) influx was also suppressed when culture medium was deprived of Ca(2+) confirming the specificity of the measure. **In conclusion, these data suggest that thimerosal induced U937 activation via oxidative stress from mitochondrial stores and mitochondrial membrane depolarization with a primordial effect of thiol groups.** A cross-talk between ROS and Ca(2+) influx was demonstrated.

81. What's going on? The question of time trends in autism.

Public Health Rep. 2004 Nov-Dec;119(6):536-51.

Blaxill MF.

Abstract

Increases in the reported prevalence of autism and autistic spectrum disorders in recent years have fueled concern over possible environmental causes. The author reviews the available survey literature and finds evidence of large increases in prevalence in both the United States and the United Kingdom that cannot be explained by changes in diagnostic criteria or improvements in case ascertainment. Incomplete ascertainment of autism cases in young child populations is the largest source of predictable bias in prevalence surveys; however, this bias has, if anything, worked against the detection of an upward trend in recent surveys. Comparison of autism rates by year of birth for specific geographies provides the strongest basis for trend assessment. Such comparisons show large recent increases in rates of autism and autistic spectrum disorders in both the U.S. and the U.K. Reported rates of autism in the United States increased from < 3 per 10,000 children in the 1970s to > 30 per 10,000 children in the 1990s, a 10-fold increase. In the United Kingdom, autism rates rose from < 10 per 10,000 in the 1980s to roughly 30 per 10,000 in the 1990s. Reported rates for the full spectrum of autistic disorders rose from the 5 to 10 per 10,000 range to the 50 to 80 per 10,000 range in the two countries. A precautionary approach suggests that the rising incidence of autism should be a matter of urgent public concern.

82. Vaccines and Autism

Laboratory medicine> september 2002> number 9> volume 33

Bernard Rimland, PhD, Woody McGinnis, MD

Autism Research Institute, San Diego, CA

Excerpt: "**Vaccinations may be one of the triggers for autism. Substantial data demonstrate immune abnormality in many autistic children consistent with impaired resistance to infection, activation of inflammatory response, and autoimmunity. Impaired resistance may predispose to vaccine injury in autism**."

83. Theoretical aspects of autism: Causes—A review

Journal of Immunotoxicology, January-March 2011, Vol. 8, No. 1 , Pages 68-79

Helen V. Ratajczak, PhD

Autism, a member of the pervasive developmental disorders (PDDs), has been increasing dramatically since its description by Leo Kanner in 1943. First estimated to occur in 4 to 5 per 10,000 children, the incidence of autism is now 1 per 110 in the United States, and 1 per 64 in the United Kingdom, with similar incidences throughout the world. Searching information from 1943 to the present in PubMed and Ovid Medline databases, this review summarizes results that

correlate the timing of changes in incidence with environmental changes. Autism could result from more than one cause, with different manifestations in different individuals that share common symptoms. Documented causes of autism include genetic mutations and/or deletions, viral infections, and encephalitis following vaccination. **Therefore, autism is the result of genetic defects and/or inflammation of the brain. The inflammation could be caused by a defective placenta, immature blood-brain barrier, the immune response of the mother to infection while pregnant, a premature birth, encephalitis in the child after birth, or a toxic environment.**

84. Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants

The Journal of Pediatrics, Volume 136, Issue 5, May 2000, Pages 679–681

Gregory V. Stajich, PharmD, Gaylord P. Lopez, PharmD, ABAT, Sokei W. Harry, MBBS, MPH, William R. Sexson, MD
Mercer University, Southern School of Pharmacy, Atlanta, Georgia; Georgia Poison Center, Grady Health System, Atlanta; Georgia Poison Center, Georgia Health System, Atlanta and Emory University, School of Medicine, Atlanta, Georgia.

Thimerosal, a derivative of mercury, is used as a preservative in hepatitis B vaccines. We measured total mercury levels before and after the administration of this vaccine in 15 preterm and 5 term infants. Comparison of pre- and post-vaccination mercury levels showed a significant increase in both preterm and term infants after vaccination. **Additionally, post-vaccination mercury levels were significantly higher in preterm infants as compared with term infants.** Because mercury is known to be a potential neurotoxin to infants, further study of its pharmacodynamics is warranted.

85. Infants born late/moderately preterm are at increased risk for a positive autism screen at 2 years of age.

J Pediatr. 2015 Feb;166(2):269-75.e3. doi: 10.1016/j.jpeds.2014.10.053. Epub 2014 Dec 2.

Guy A1, Seaton SE2, Boyle EM2, Draper ES2, Field DJ2, Manktelow BN2, Marlow N3, Smith LK2, Johnson S4.

1Department of Health Sciences, University of Leicester, Leicester, United Kingdom; School of Psychology, University of Warwick, Coventry, United Kingdom.
2Department of Health Sciences, University of Leicester, Leicester, United Kingdom.

3Department of Academic Neonatology, Institute for Women's Health, University College London, London, United Kingdom.
4Department of Health Sciences, University of Leicester, Leicester, United Kingdom. Electronic address: sjj19@le.ac.uk.

Abstract
OBJECTIVES:
To assess the prevalence of positive screens using the Modified Checklist for Autism in Toddlers (M-CHAT) questionnaire and follow-up interview in late and moderately preterm (LMPT; 32-36 weeks) infants and term-born controls.
STUDY DESIGN:
Population-based prospective cohort study of 1130 LMPT and 1255 term-born infants. Parents completed the M-CHAT questionnaire at 2-years corrected age. Parents of infants with positive questionnaire screens were followed up with a telephone interview to clarify failed items. The M-CHAT questionnaire was re-scored, and infants were classified as true or false positives. Neurosensory, cognitive, and behavioral outcomes were assessed using parent report.
RESULTS:
Parents of 634 (57%) LMPT and 761 (62%) term-born infants completed the M-CHAT questionnaire. LMPT infants had significantly higher risk of a positive questionnaire screen compared with controls (14.5% vs 9.2%; relative risk [RR] 1.58; 95% CI 1.18, 2.11). After follow-up, significantly more LMPT infants than controls had a true positive screen (2.4% vs 0.5%; RR 4.52; 1.51, 13.56). This remained significant after excluding infants with neurosensory impairments (2.0% vs 0.5%; RR 3.67; 1.19, 11.3).
CONCLUSIONS:
LMPT infants are at significantly increased risk for positive autistic screen. An M-CHAT follow-up interview is essential as screening for autism spectrum disorders is especially confounded in preterm populations. Infants with false positive screens are at risk for cognitive and behavioral problems.

86. Preterm birth and mortality and morbidity: a population-based quasi-experimental study.

JAMA Psychiatry. 2013 Nov;70(11):1231-40. doi: 10.1001/jamapsychiatry.2013.2107.

D'Onofrio BM1, Class QA, Rickert ME, Larsson H, Långström N, Lichtenstein P.

Department of Psychological and Brain Sciences, Indiana University-Bloomington.

Abstract
IMPORTANCE:
Preterm birth is associated with increased mortality and morbidity. However, previous studies have been unable to rigorously examine whether confounding factors cause these associations rather than the harmful effects of being born preterm.

OBJECTIVE:
To estimate the extent to which the associations between early gestational age and offspring mortality and morbidity are the result of confounding factors by using a quasi-experimental design, the sibling-comparison approach, and by controlling for statistical covariates that varied within families.
DESIGN, SETTING, AND PARTICIPANTS:
A population-based cohort study, combining Swedish registries to identify all individuals born in Sweden from 1973 to 2008 (3,300,708 offspring of 1,736,735 mothers) and link them with multiple outcomes.
MAIN OUTCOMES AND MEASURES:
Offspring mortality (during infancy and throughout young adulthood) and psychiatric (psychotic or bipolar disorder, autism, attention-deficit/hyperactivity disorder, suicide attempts, substance use, and criminality), academic (failing grades and educational attainment), and social (partnering, parenthood, low income, and social welfare benefits) outcomes through 2009.
RESULTS:
In the population, there was a dose-response relationship between early gestation and the outcome measures. **For example, extreme preterm birth (23-27 weeks of gestation) was associated with infant mortality (odds ratio, 288.1; 95% CI, 271.7-305.5), autism (hazard ratio [HR], 3.2; 95% CI, 2.6-4.0)**, low educational attainment (HR, 1.7; 1.5-2.0), and social welfare benefits (HR, 1.3; 1.2-1.5) compared with offspring born at term. The associations between early gestation and mortality and psychiatric morbidity generally were robust when comparing differentially exposed siblings and controlling for statistical covariates, whereas the associations with academic and some social problems were greatly or completely attenuated in the fixed-effects models.
CONCLUSIONS AND RELEVANCE:
The mechanisms responsible for the associations between preterm birth and mortality and morbidity are outcome-specific. Associations between preterm birth and mortality and psychiatric morbidity are largely independent of shared familial confounds and measured covariates, consistent with a causal inference. However, some associations, particularly predicting suicide attempt, educational attainment, and social welfare benefits, are the result of confounding factors. The findings emphasize the importance of both reducing preterm birth and providing wraparound services to all siblings in families with an offspring born preterm.

87. Ancestry of pink disease (infantile acrodynia) identified as a risk factor for autism spectrum disorders.

J Toxicol Environ Health A. 2011 Sep 15;74(18):1185-94.
Shandley K, Austin DW.

Swinburne Autism Bio-Research Initiative (SABRI), Brain and Psychological Sciences Research Centre , Swinburne University of Technology , Hawthorn , Victoria , Australia.

Abstract

Pink disease (infantile acrodynia) was especially prevalent in the first half of the 20th century. Primarily attributed to exposure to mercury (Hg) commonly found in teething powders, the condition was developed by approximately 1 in 500 exposed children. The differential risk factor was identified as an idiosyncratic sensitivity to Hg. Autismspectrum disorders (ASD) have also been postulated to be produced by Hg. Analogous to the pink disease experience, Hg exposure is widespread yet only a fraction of exposed children develop an ASD, suggesting sensitivity to Hg may also be present in children with an ASD. The objective of this study was to test the hypothesis that individuals with a known hypersensitivity to Hg (pink disease survivors) may be more likely to have descendants with an ASD. Five hundred and twenty-two participants who had previously been diagnosed with pink disease completed a survey on the health outcomes of their descendants. The prevalence rates of ASD and a variety of other clinical conditions diagnosed in childhood (attention deficit hyperactivity disorder, epilepsy, Fragile X syndrome, and Down syndrome) were compared to well-established general population prevalence rates. The results showed the prevalence rate of ASD among the grandchildren of pink disease survivors (1 in 22) to be significantly higher than the comparable general population prevalence rate (1 in 160). **The results support the hypothesis that Hg sensitivity may be a heritable/genetic risk factor for ASD.**

88. Risk Factors for Autistic Regression: Results of an Ambispective Cohort Study.

J Child Neurol. 2012 Jan 30. [Epub ahead of print]

Zhang Y, Xu Q, Liu J, Li SC, Xu X., Department of Child Health Care, Children's Hospital of Fudan University,Shanghai, China.

Abstract
A subgroup of children diagnosed with autism experience developmental regression featured by a loss of previously acquired abilities. The pathogeny of autistic regression is unknown, although many risk factors likely exist. To better characterize autistic regression and investigate the association between autistic regression and potential influencing factors in Chinese autistic children, we conducted an ambispective study with a cohort of 170 autistic subjects. **Analyses by multiple logistic regression showed significant correlations between autistic regression and febrile seizures (OR = 3.53, 95% CI = 1.17-10.65, P = .025)**, as well as with a family history of neuropsychiatric disorders (OR = 3.62, 95% CI = 1.35-9.71, P = .011). **This study suggests that febrile seizures and family history of neuropsychiatric disorders are correlated with autistic regression.**

89. MMR vaccination and febrile seizures: evaluation of susceptible subgroups and long-term prognosis.

Vestergaard M1, Hviid A, Madsen KM, Wohlfahrt J, Thorsen P, Schendel D,

Melbye M, Olsen J.

JAMA. 2004 Jul 21;292(3):351-7.

Abstract
CONTEXT:
The rate of febrile seizures increases following measles, mumps, and rubella (MMR) vaccination but it is unknown whether the rate varies according to personal or family history of seizures, perinatal factors, or socioeconomic status. Furthermore, little is known about the long-term outcome of febrile seizures following vaccination.
OBJECTIVES:
To estimate incidence rate ratios (RRs) and risk differences of febrile seizures following MMR vaccination within subgroups of children and to evaluate the clinical outcome of febrile seizures following vaccination.
DESIGN, SETTING, AND PARTICIPANTS:
A population-based cohort study of all children born in Denmark between January 1, 1991, and December 31, 1998, who were alive at 3 months; 537,171 children were followed up until December 31, 1999, by using data from the Danish Civil Registration System and 4 other national registries.
MAIN OUTCOME MEASURES:
Incidence of first febrile seizure, recurrent febrile seizures, and subsequent epilepsy.
RESULTS:
A total of 439,251 children (82%) received MMR vaccination and 17,986 children developed febrile seizures at least once; 973 of these febrile seizures occurred within 2 weeks of MMR vaccination. The RR of febrile seizures increased during the 2 weeks following MMR vaccination (2.75; 95% confidence interval [CI], 2.55-2.97), and thereafter was close to the observed RR for nonvaccinated children. The RR did not vary significantly in the subgroups of children that had been defined by their family history of seizures, perinatal factors, or socioeconomic status. At 15 to 17 months, the risk difference of febrile seizures within 2 weeks following MMR vaccination was 1.56 per 1000 children overall (95% CI, 1.44-1.68), 3.97 per 1000 (95% CI, 2.90-5.40) for siblings of children with a history of febrile seizures, and 19.47 per 1000 (95% CI, 16.05-23.55) for children with a personal history of febrile seizures. Children with febrile seizures following MMR vaccinations had a slightly increased rate of recurrent febrile seizures (RR, 1.19; 95% CI, 1.01-1.41) but no increased rate of epilepsy (RR, 0.70; 95% CI, 0.33-1.50) compared with children who were nonvaccinated at the time of their first febrile seizure.
CONCLUSIONS:
MMR vaccination was associated with a transient increased rate of febrile seizures but the risk difference was small even in high-risk children. The long-term rate of epilepsy was not increased in children who had febrile seizures following vaccination compared with children who had febrile seizures of a different etiology.

90. Common variants associated with general and MMR vaccine–related febrile seizures

Bjarke Feenstra, Björn Pasternak, Frank Geller, Lisbeth Carstensen, Tongfei Wang, Fen Huang, Jennifer L Eitson, Mads V Hollegaard, Henrik Svanström, Mogens Vestergaard, David M Hougaard, John W Schoggins, Lily Yeh Jan, Mads Melbye & Anders Hviid

Nature Genetics (2014) doi:10.1038/ng.3129

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Abstract
Febrile seizures represent a serious adverse event following measles, mumps and rubella (MMR) vaccination. We conducted a series of genome-wide association scans comparing children with MMR-related febrile seizures, children with febrile seizures unrelated to vaccination and controls with no history of febrile seizures. Two loci were distinctly associated with MMR-related febrile seizures, harboring the interferon-stimulated gene IFI44L (rs273259: $P = 5.9 \times 10^{-12}$ versus controls, $P = 1.2 \times 10^{-9}$ versus MMR-unrelated febrile seizures) and the measles virus receptor CD46 (rs1318653: $P = 9.6 \times 10^{-11}$ versus controls, $P = 1.6 \times 10^{-9}$ versus MMR-unrelated febrile seizures). Furthermore, four loci were associated with febrile seizures in general, implicating the sodium channel genes SCN1A (rs6432860: $P = 2.2 \times 10^{-16}$) and SCN2A (rs3769955: $P = 3.1 \times 10^{-10}$), a TMEM16 family gene (ANO3; rs114444506: $P = 3.7 \times 10^{-20}$) and a region associated with magnesium levels (12q21.33; rs11105468: $P = 3.4 \times 10^{-11}$). Finally, we show the functional relevance of ANO3 (TMEM16C) with electrophysiological experiments in wild-type and knockout rats.

91. Adverse events following 12 and 18 month vaccinations: a population-based, self-controlled case series analysis.

PLoS One. 2011;6(12):e27897. Epub 2011 Dec 12.

Wilson K, Hawken S, Kwong JC, Deeks S, Crowcroft NS, Van Walraven C, Potter BK, Chakraborty P, Keelan J, Pluscauskas M, Manuel D. Department of Medicine, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Canada. kwilson@ohri.ca

Abstract
BACKGROUND:
Live vaccines have distinct safety profiles, potentially causing systemic reactions one to 2 weeks after administration. In the province of Ontario, Canada, live MMR vaccine is currently recommended at age 12 months and 18 months.

METHODS:
Using the self-controlled case series design we examined 271,495 12 month

vaccinations and 184,312 18 month vaccinations to examine the relative incidence of the composite endpoint of emergency room visits or hospital admissions in consecutive one day intervals following vaccination. These were compared to a control period 20 to 28 days later. In a post-hoc analysis we examined the reasons for emergency room visits and the average acuity score at presentation for children during the at-risk period following the 12 month vaccine.

RESULTS:
Four to 12 days post 12 month vaccination, children had a 1.33 (1.29-1.38) increased relative incidence of the combined endpoint compared to the control period, or at least one event during the risk interval for every 168 children vaccinated. Ten to 12 days post 18 month vaccination, the relative incidence was 1.25 (95%, 1.17-1.33) which represented at least one excess event for every 730 children vaccinated. The primary reason for increased events was statistically significant elevations in emergency room visits following all vaccinations. There were non-significant increases in hospital admissions. **There were an additional 20 febrile seizures for every 100,000 vaccinated at 12 months.**

CONCLUSIONS:
There are significantly elevated risks of primarily emergency room visits approximately one to two weeks following 12 and 18 month vaccination. Future studies should examine whether these events could be predicted or prevented.

92. Reduced GABAergic Action in the Autistic Brain.

Curr Biol. 2015 Dec 16. pii: S0960-9822(15)01413-X. doi: 10.1016/j.cub.2015.11.019.

Robertson CE1, Ratai EM2, Kanwisher N3.

1Harvard Society of Fellows, Harvard University, Cambridge, MA 02138, USA; McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02138, USA. Electronic address: carolinerobertson@fas.harvard.edu.
2Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA.
3McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02138, USA.

Abstract
An imbalance between excitatory/inhibitory neurotransmission has been posited as a central characteristic of the neurobiology of autism [1], inspired in part by the striking prevalence of seizures among individuals with the disorder [2]. Evidence supporting this hypothesis has specifically implicated the signaling pathway of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), in this putative imbalance: GABA receptor genes have been associated with autism in linkage and copy number variation studies [3-7], fewer GABA receptor subunits have been observed in the post-mortem tissue of autistic individuals [8, 9], and

GABAergic signaling is disrupted across heterogeneous mouse models of autism [10]. Yet, empirical evidence supporting this hypothesis in humans is lacking, leaving a gulf between animal and human studies of the condition. Here, we present a direct link between GABA signaling and autistic perceptual symptomatology. We first demonstrate a robust, replicated autistic deficit in binocular rivalry [11], a basic visual function that is thought to rely on the balance of excitation/inhibition in visual cortex [12-15]. **Then, using magnetic resonance spectroscopy, we demonstrate a tight linkage between binocular rivalry dynamics in typical participants and both GABA and glutamate levels in the visual cortex. Finally, we show that the link between GABA and binocular rivalry dynamics is completely and specifically absent in autism.** These results suggest a disruption in inhibitory signaling in the autistic brain and forge a translational path between animal and human models of the condition.

93. Administration of thimerosal to infant rats increases overflow of glutamate and aspartate in the prefrontal cortex: protective role of dehydroepiandrosterone sulfate.

Neurochem Res. 2012 Feb;37(2):436-47. Epub 2011 Oct 21.

Duszczyk-Budhathoki M, Olczak M, Lehner M, Majewska MD. Marie Curie Chairs Program, Department of Pharmacology and Physiology of Nervous System, Institute of Psychiatry and Neurology, 02-957, Warsaw, Poland.

Abstract
Thimerosal, a mercury-containing vaccine preservative, is a suspected factor in the etiology of neurodevelopmental disorders. We previously showed that its administration to infant rats causes behavioral, neurochemical and neuropathological abnormalities similar to those present in autism. Here we examined, using microdialysis, the effect of thimerosal on extracellular levels of neuroactive amino acids in the rat prefrontal cortex (PFC). Thimerosal administration (4 injections, i.m., 240 μg Hg/kg on postnatal days 7, 9, 11, 15) induced lasting changes in amino acid overflow: an increase of glutamate and aspartate accompanied by a decrease of glycine and alanine; measured 10-14 weeks after the injections. Four injections of thimerosal at a dose of 12.5 μg Hg/kg did not alter glutamate and aspartate concentrations at microdialysis time (but based on thimerosal pharmacokinetics, could have been effective soon after its injection). Application of thimerosal to the PFC in perfusion fluid evoked a rapid increase of glutamate overflow. Coadministration of the neurosteroid, dehydroepiandrosterone sulfate (DHEAS; 80 mg/kg; i.p.) prevented the thimerosal effect on glutamate and aspartate; the steroid alone had no influence on these amino acids. Coapplication of DHEAS with thimerosal in perfusion fluid also blocked the acute action of thimerosal on glutamate. In contrast, DHEAS alone reduced overflow of glycine and alanine, somewhat potentiating the thimerosal effect on these amino acids. **Since excessive accumulation of extracellular glutamate is linked with excitotoxicity, our data imply that neonatal exposure to thimerosal-containing vaccines might induce excitotoxic brain injuries, leading to neurodevelopmental disorders.** DHEAS

may partially protect against mercurials-induced neurotoxicity.

94. Neonatal Administration of Thimerosal Causes Persistent Changes in Mu Opioid Receptors in the Rat Brain

Neurochem Res. 2010 November; 35(11): 1840–1847.

Mieszko Olczak, Michalina Duszczyk, Pawel Mierzejewski, Teresa Bobrowicz, and Maria Dorota Majewska1, Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Sobieskiego 9 str., 02-957 Warsaw, Poland, Department of Forensic Medicine, Medical University of Warsaw, Oczki 1 str., 02-007 Warsaw, Poland, Department of Neuropathology, Institute of Psychiatry and Neurology, 02-957 Warsaw, Poland, Department of Biology and Environmental Science, University of Cardinal Stefan Wyszynski, Wóycickiego Str. 1/3, 01-815 Warsaw, Poland

Abstract
Thimerosal added to some pediatric vaccines is suspected in pathogenesis of several neurodevelopmental disorders. Our previous study showed that thimerosal administered to suckling rats causes persistent, endogenous opioid-mediated hypoalgesia. Here we examined, using immunohistochemical staining technique, the density of μ-opioid receptors (MORs) in the brains of rats, which in the second postnatal week received four i.m. injections of thimerosal at doses 12, 240, 1,440 or 3,000 μg Hg/kg. The periaqueductal gray, caudate putamen and hippocampus were examined. Thimerosal administration caused dose-dependent statistically significant increase in MOR densities in the periaqueductal gray and caudate putamen, but decrease in the dentate gyrus, where it was accompanied by the presence of degenerating neurons and loss of synaptic vesicle marker (synaptophysin). **These data document that exposure to thimerosal during early postnatal life produces lasting alterations in the densities of brain opioid receptors along with other neuropathological changes, which may disturb brain development.**

95. Unanswered Questions: A Review of Compensated Cases of Vaccine-Induced Brain Injury

Pace Environmental Law Review, vol. 28, no. 2, 2011

Mary Holland, Louis Conte, Robert Krakow and Lisa Colin

Executive Summary
In 1986, Congress created the Vaccine Injury Compensation Program (VICP) under the National Childhood Vaccine Injury Act (1986 Law). This Program has original jurisdiction for children’s claims of vaccine injury. Because almost all children receive multiple vaccinations for daycare and school, it is critically important that the Program provides fundamental fairness, due process and

transparency.

This empirical investigation, published in a peer-reviewed law journal, examines claims that the VICP compensated for vaccine-induced encephalopathy and seizure disorder. The VICP has compensated approximately 2,500 claims of vaccine injury since the inception of the program. This study found 83 cases of acknowledged vaccine-induced brain damage that include autism, a disorder that affects speech, social communication and behavior. In 21 published cases of the Court of Federal Claims, which administers the VICP, the Court stated that the petitioners had autism or described autism unambiguously. In 62 remaining cases, the authors identified settlement agreements where Health and Human Services (HHS) compensated children with vaccine-induced brain damage, who also have autism or an autism spectrum disorder.

Parents reported the existence of autism in telephone interviews and supplied supplemental materials including medical diagnoses, school records, and completed, standard autism screening questionnaires to verify their reports. In 39 of the 83 cases, or 47% of the cases of vaccine injury reviewed, there is confirmation of autism or autism spectrum disorder beyond parental report.

This finding of autism in compensated cases of vaccine injury is significant. U.S. government spokespeople have been asserting no vaccine-autism link for more than a decade. This finding calls into question the decisions of the Court of Federal Claims in the Omnibus Autism Proceeding in 2009 and 2010 and the statement of Health and Human Services on its website that "HHS has never concluded in any case that autism was caused by vaccination."

Using publicly available information, the investigation shows that the VICP has been compensating cases of vaccine-induced brain damage associated with autism for more than twenty years. This investigation suggests that officials at HHS, the Department of Justice and the Court of Federal Claims may have been aware of this association but failed to publicly disclose it.

The study calls on Congress to thoroughly investigate the VICP, including a medical investigation of compensated claims of vaccine injury. This investigation calls on Congress to get answers to these critically important unanswered questions.

96. Integrating experimental (in vitro and in vivo) neurotoxicity studies of low-dose thimerosal relevant to vaccines.

Neurochem Res. 2011 Jun;36(6):927-38. doi: 10.1007/s11064-011-0427-0. Epub 2011 Feb 25.

Dórea JG, Faculty of Health Sciences, Universidade de Brasília, CP 04322, 70919-970, Brasília, DF, Brazil. dorea@rudah.com.br

Abstract
There is a need to interpret neurotoxic studies to help deal with uncertainties surrounding pregnant mothers, newborns and young children who must receive repeated doses of Thimerosal-containing vaccines (TCVs). This review integrates information derived from emerging experimental studies (in vitro and in vivo) of low-dose Thimerosal (sodium ethyl mercury thiosalicylate). Major databases (PubMed and Web-of-science) were searched for in vitro and in vivo experimental studies that addressed the effects of low-dose Thimerosal (or ethylmercury) on neural tissues and animal behaviour. Information extracted from studies indicates that: (a) activity of low doses of Thimerosal against isolated human and animal brain cells was found in all studies and is consistent with Hg neurotoxicity; (b) the neurotoxic effect of ethylmercury has not been studied with co-occurring adjuvant-Al in TCVs; (c) animal studies have shown that exposure to Thimerosal-Hg can lead to accumulation of inorganic Hg in brain, and that (d) doses relevant to TCV exposure possess the potential to affect human neuro-development. Thimerosal at concentrations relevant for infants' exposure (in vaccines) is toxic to cultured human-brain cells and to laboratory animals. The persisting use of TCV (in developing countries) is counterintuitive to global efforts to lower Hg exposure and to ban Hg in medical products; its continued use in TCV requires evaluation of a sufficiently nontoxic level of ethylmercury compatible with repeated exposure (co-occurring with adjuvant-Al) during early life.

97. Hepatitis B vaccine induces apoptotic death in Hepa1-6 cells

Apoptosis. 2012 Jan 17. Hamza H, Cao J, Li X, Li C, Zhu M, Zhao S.

Key Lab of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China, Heyam68_hamza@yahoo.com.

Abstract
Vaccines can have adverse side-effects, and these are predominantly associated with the inclusion of chemical additives such as aluminum hydroxide adjuvant. The objective of this study was to establish an in vitro model system amenable to mechanistic investigations of cytotoxicity induced by hepatitis B vaccine, and to investigate the mechanisms of vaccine-induced cell death. The mouse liver hepatoma cell line Hepa1-6 was treated with two doses of adjuvanted (aluminium hydroxide) hepatitis B vaccine (0.5 and 1 µg protein per ml) and cell integrity was measured after 24, 48 and 72 h. Hepatitis B vaccine exposure increased cell apoptosis as detected by flow cytometry and TUNEL assay. Vaccine exposure was accompanied by significant increases in the levels of activated caspase 3, a key effector caspase in the apoptosis cascade. Early transcriptional events were detected by qRT-PCR. We report that hepatitis B vaccine exposure resulted in significant upregulation of the key genes encoding caspase 7, caspase 9,

Inhibitor caspase-activated DNase (ICAD), Rho-associated coiled-coil containing protein kinase 1 (ROCK-1), and Apoptotic protease activating factor 1 (Apaf-1). Upregulation of cleaved caspase 3,7 were detected by western blot in addition to Apaf-1 and caspase 9 expressions argues that cell death takes place via the intrinsic apoptotic pathway in which release of cytochrome c from the mitochondria triggers the assembly of a caspase activation complex. **We conclude that exposure of Hepa1-6 cells to a low dose of adjuvanted hepatitis B vaccine leads to loss of mitochondrial integrity, apoptosis induction, and cell death, apoptosis effect was observed also in C2C12 mouse myoblast cell line after treated with low dose of vaccine (0.3, 0.1, 0.05 μg/ml)**. In addition In vivo apoptotic effect of hepatitis B vaccine was observed in mouse liver.

98. Thimerosal Induces Apoptosis in a Neuroblastoma Model via the cJun N-Terminal Kinase Pathway.

Toxicological Sciences 92 (1). 246-253

ML Herdman, A Marcelo, Y Huang, RM Niles, Dhar S & Kiningham KK. (2006).

Department of Pharmacology, Joan C. Edwards School of Medicine, 1542 Spring Valley Drive, Marshall University, Huntington, WV USA

EXCERPT: In recent years, controversy has surrounded the use of thimerosal in vaccines as mercury is a known neurotoxin and nephrotoxin. Since the controversy began in the late 1990's, much of the thimerosal has been removed from vaccines administered to children in the United States. However, it remains in some, such as the influenza vaccine, and is added to multidose vials used in countries around the world. Studies concentrating on thimerosal-induced neurotoxicity are limited, and exposure guidelines, such as those set by the Food and Drug Administration, are based on research with methylmercury. Interestingly, some in vitro and in vivo studies suggest that ethylmercury may react differently than methylmercury (Aschner and Aschner, 1990; Harry et al., 2004; Magos et al., 1985). Few studies with thimerosal have focused on determining specific signaling pathways involved in neurotoxicity. Establishing these pathways may be an important step in discovering methods of alleviating toxic outcomes in patients exposed to thimerosal….Our study is the first demonstration that thimerosal can induce the activation of JNK and AP-1 in the SK-N-SH neuroblastoma cell line. We showed that activation of cJun and AP-1 transcriptional activity following thimerosal treatment does not appear to be involved in the induction of apoptosis, as demonstrated with the studies using the cJun dominant negative. Furthermore, we were able to show that JNK is an essential upstream component of this pathway through the use of the JNK inhibitor SP600125. This compound was able to attenuate activation of downstream components of mitochondrial-mediated cell death and subsequently protect the cells from apoptosis. These results are significant because identifying

specific signaling pathways activated in response to thimerosal exposure presents pharmacological targets for attenuating potential toxicity in patients exposed to thimerosal-containing products.

99. Maternal thimerosal exposure results in aberrant cerebellar oxidative stress, thyroid hormone metabolism, and motor behavior in rat pups; sex- and strain-dependent effects.

Cerebellum. 2012 Jun;11(2):575-86. doi: 10.1007/s12311-011-0319-5.

Sulkowski ZL, Chen T, Midha S, Zavacki AM, Sajdel-Sulkowska EM, Department of Psychiatry, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA.

Abstract
Methylmercury (Met-Hg) and ethylmercury (Et-Hg) are powerful toxicants with a range of harmful neurological effects in humans and animals. While Met-Hg is a recognized trigger of oxidative stress and an endocrine disruptor impacting neurodevelopment, the developmental neurotoxicity of Et-Hg, a metabolite of thimerosal (TM), has not been explored. We hypothesized that TM exposure during the perinatal period impairs central nervous system development, and specifically the cerebellum, by the mechanism involving oxidative stress. To test this, spontaneously hypertensive rats (SHR) or Sprague-Dawley (SD) rat dams were exposed to TM (200 μg/kg body weight) during pregnancy (G10-G15) and lactation (P5-P10). Male and female neonates were evaluated for auditory and motor function; cerebella were analyzed for oxidative stress and thyroid metabolism. TM exposure resulted in a delayed startle response in SD neonates and decreased motor learning in SHR male (22.6%), in SD male (29.8%), and in SD female (55.0%) neonates. TM exposure also resulted in a significant increase in cerebellar levels of the oxidative stress marker 3-nitrotyrosine in SHR female (35.1%) and SD male (14.0%) neonates. The activity of cerebellar type 2 deiodinase, responsible for local intra-brain conversion of thyroxine to the active hormone, 3',3,5-triiodothyronine (T3), was significantly decreased in TM-exposed SHR male (60.9%) pups. This coincided with an increased (47.0%) expression of a gene negatively regulated by T3, Odf4 suggesting local intracerebellar T3 deficiency. **Our data thus demonstrate a negative neurodevelopmental impact of perinatal TM exposure which appears to be both strain- and sex-dependent.**

100. The rise in autism and the role of age at diagnosis.

Epidemiology. 2009 Jan;20(1):84-90. doi: 10.1097/EDE.0b013e3181902d15.

Hertz-Picciotto I, Delwiche L., Department of Public Health Sciences, University of California, Davis, California 95616, USA. ihp@ucdavis.edu

Abstract
BACKGROUND:
Autism prevalence in California, based on individuals eligible for state-funded services, rose throughout the 1990s. The extent to which this trend is explained by changes in age at diagnosis or inclusion of milder cases has not been previously evaluated.
METHODS:
Autism cases were identified from 1990 through 2006 in databases of the California Department of Developmental Services, which coordinates services for individuals with specific developmental disorders. The main outcomes were population incident cases younger than age 10 years for each quarter, cumulative incidence by age and birth year, age-specific incidence rates stratified by birth year, and proportions of diagnoses by age across birth years.
RESULTS:
Autism incidence in children rose throughout the period. Cumulative incidence to 5 years of age per 10,000 births rose consistently from 6.2 for 1990 births to 42.5 for 2001 births. Age-specific incidence rates increased most steeply for 2- and 3-year olds. The proportion diagnosed by age 5 years increased only slightly, from 54% for 1990 births to 61% for 1996 births. Changing age at diagnosis can explain a 12% increase, and inclusion of milder cases, a 56% increase.
CONCLUSIONS:
Autism incidence in California shows no sign yet of plateauing. Younger ages at diagnosis, differential migration, changes in diagnostic criteria, and inclusion of milder cases do not fully explain the observed increases. Other artifacts have yet to be quantified, and as a result, the extent to which the continued rise represents a true increase in the occurrence of autism remains unclear.

101. Slow CCL2-dependent translocation of biopersistent particles from muscle to brain

Zakir Khan[1,2], Christophe Combadière[3,4,5], François-Jérôme Authier[1,2,6], Valérie Itier[1,11,2], François Lux[7,8], Christopher Exley[9], Meriem Mahrouf-Yorgov[1,11,2], Xavier Decrouy[1,2], Philippe Moretto[10], Olivier Tillement[7,8], Romain K Gherardi[1,12,2,6]*† and Josette Cadusseau[1,11,12,2]*†

Author Affiliations

1 Inserm, U955, 8 rue du Général Sarrail, Créteil, 94010, France

2 Université Paris Est, Faculté de Médecine, 8 rue du Général Sarrail, Créteil, 94010, France

3 Inserm, UMR-S 945, 91 Boulevard de l'Hôpital, Paris, 75013, France

4 Université Pierre et Marie Curie, Faculté de Médecine, 11 Boulevard de l'Hôpital, Paris, 75013, France

5 AP-HP, Groupe Hospitalier Pitié-Salpétrière, Service d'Immunologie, 11

Boulevard de l'Hôpital, Paris, 75013, France

6 AP-HP, Hôpital H. Mondor - A. Chenevier, Service d'Histologie, Centre de Référence Neuromusculaire GNMH, 51 Avenue du Maréchal de Lattre de Tassigny, Créteil, 94000, France

7 CNRS UMR 5620, Laboratoire de Physico-Chimie des Matériaux Luminescents, 2 rue Victor Grignard, Villeurbanne, 69622, France

8 Université Claude Bernard Lyon 1, 2 rue Victor Grignard, Villeurbanne, 69622, France

9 The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, ST5 5BG, UK

10 CNRS UMR 5797, Centre d'Etudes Nucléaires de Bordeaux Gradignan, Allée du haut Vignaud, Gradignan, 33175, France

11 Faculté des Sciences et Technologie, UPEC, 61 Avenue du Général de Gaulle, Créteil, France

12 IMRB Team 10, Faculté de Médecine, 8 rue du Général Sarrail, Créteil, F-94010, France

BMC Medicine 2013, 11:99 doi:10.1186/1741-7015-11-99, 4 April 2013

Abstract

Background
Long-term biodistribution of nanomaterials used in medicine is largely unknown. This is the case for alum, the most widely used vaccine adjuvant, which is a nanocrystalline compound spontaneously forming micron/submicron-sized agglomerates. Although generally well tolerated, alum is occasionally detected within monocyte-lineage cells long after immunization in presumably susceptible individuals with systemic/neurologic manifestations or autoimmune (inflammatory) syndrome induced by adjuvants (ASIA).
Methods:
On the grounds of preliminary investigations in 252 patients with alum-associated ASIA showing both a selective increase of circulating CCL2, the major monocyte chemoattractant, and a variation in the CCL2 gene, we designed mouse experiments to assess biodistribution of vaccine-derived aluminum and of alum-particle fluorescent surrogates injected in muscle. Aluminum was detected in tissues by Morin stain and particle induced X-ray emission) (PIXE) Both 500 nm fluorescent latex beads and vaccine alum agglomerates-sized nanohybrids (Al-Rho) were used.
Results:
Intramuscular injection of alum-containing vaccine was associated with the appearance of aluminum deposits in distant organs, such as spleen and brain where they were still detected one year after injection. Both fluorescent materials

injected into muscle translocated to draining lymph nodes (DLNs) and thereafter were detected associated with phagocytes in blood and spleen. Particles linearly accumulated in the brain up to the six-month endpoint; they were first found in perivascular CD11b+ cells and then in microglia and other neural cells. DLN ablation dramatically reduced the biodistribution. Cerebral translocation was not observed after direct intravenous injection, but significantly increased in mice with chronically altered blood-brain-barrier. Loss/gain-of-function experiments consistently implicated CCL2 in systemic diffusion of Al-Rho particles captured by monocyte-lineage cells and in their subsequent neurodelivery. Stereotactic particle injection pointed out brain retention as a factor of progressive particle accumulation.

Conclusion

Nanomaterials can be transported by monocyte-lineage cells to DLNs, blood and spleen, and, similarly to HIV, may use CCL2-dependent mechanisms to penetrate the brain. This occurs at a very low rate in normal conditions explaining good overall tolerance of alum despite its strong neurotoxic potential. However, continuously escalating doses of this poorly biodegradable adjuvant in the population may become insidiously unsafe, especially in the case of overimmunization or immature/altered blood brain barrier or high constitutive CCL-2 production.

102. Thimerosal and autism? A plausible hypothesis that should not be dismissed.

Med Hypotheses. 2004;62(5):788-94.

Blaxill MF, Redwood L, Bernard S.

Abstract

The autism-mercury hypothesis first described by Bernard et al. has generated much interest and controversy. The Institute of Medicine (IOM) reviewed the connection between mercury-containing vaccines and neurodevelopmental disorders, including autism. They concluded that the hypothesis was biologically plausible but that there was insufficient evidence to accept or reject a causal connection and recommended a comprehensive research program. Without citing new experimental evidence, a number of observers have offered opinions on the subject, some of which reject the IOM's conclusions. In a recent review, Nelson and Bauman argue that a link between the preservative thimerosal, the source of the mercury in childhood vaccines, is improbable. In their defense of thimerosal, these authors take a narrow view of the original hypothesis, provide no new evidence, and rely on selective citations and flawed reasoning. We provide evidence here to refute the Nelson and Bauman critique and to defend the autism-mercury hypothesis.

103. Autism Spectrum Disorders in Relation to Distribution of Hazardous Air Pollutants in the SF Bay Area

Environmental Health Perspectives – Vol. 114 No. 9, September, 2006

Gayle Windham, Div. of Environmental and Occupational Disease Control, California Department of Health Services

284 ASD children & 657 controls, born in 1994 in Bay Area, were assigned exposure levels by birth tract for 19 chemicals. Risks for autism were elevated by 50% in tracts with the highest chlorinated solvents and heavy metals. The highest risk compounds were mercury, cadmium, nickel, trichloroethylene, and vinyl chloride, and the risk from heavy metals was almost twice as high as solvents.

Excerpt: "Our results suggest a potential association between autism and estimated metal concentrations, and possibly solvents, in ambient air around the birth residence."

104. Environmental mercury release, special education rates, and autism disorder: an ecological study of Texas

Health Place. 2006 Jun;12(2):203-9.

Palmer RF, Blanchard S, Stein Z, Mandell D, Miller C.

University of Texas Health Science Center, San Antonio Department of Family and Community Medicine, 7703 Floyd Curl Drive, San Antonio, Texas

Abstract
The association between environmentally released mercury, special education and autism rates in Texas was investigated using data from the Texas Education Department and the United States Environmental Protection Agency. A Poisson regression analysis adjusted for school district population size, economic and demographic factors was used. There was a significant increase in the rates of special education students and autism rates associated with increases in environmentally released mercury. On average, for each 1,000 lb of environmentally released mercury, there was a 43% increase in the rate of special education services and a 61% increase in the rate of autism. The association between environmentally released mercury and special education rates were fully mediated by increased autism rates. This ecological study suggests the need for further research regarding the association between environmentally released mercury and developmental disorders such as autism. These results have implications for policy planning and cost analysis.

105. Autism spectrum disorder prevalence and proximity to industrial facilities releasing arsenic, lead or mercury.

Sci Total Environ. 2015 Dec 1;536:245-51. doi: 10.1016/j.scitotenv.2015.07.024. Epub 2015 Jul 25.

Dickerson AS1, Rahbar MH2, Han I3, Bakian AV4, Bilder DA5, Harrington RA6, Pettygrove S7, Durkin M8, Kirby RS9, Wingate MS10, Tian LH11, Zahorodny WM12, Pearson DA13, Moyé LA 3rd14, Baio J15.

1Biostatistics/Epidemiology/Research Design (BERD) Core, Center for Clinical and Translational Sciences (CCTS), University of Texas Health Science Center at Houston, Houston, TX 77030, USA. Electronic address: Aisha.S.Dickerson@uth.tmc.edu.
2Biostatistics/Epidemiology/Research Design (BERD) Core, Center for Clinical and Translational Sciences (CCTS), University of Texas Health Science Center at Houston, Houston, TX 77030, USA; Division of Epidemiology, Human Genetics, and Environmental Sciences (EHGES), University of Texas School of Public Health at Houston, University of Texas Health Science Center at Houston, Houston, TX 77030, USA. Electronic address: Mohammad.H.Rahbar@uth.tmc.edu.
3Division of Epidemiology, Human Genetics, and Environmental Sciences (EHGES), University of Texas School of Public Health at Houston, University of Texas Health Science Center at Houston, Houston, TX 77030, USA. Electronic address: Inkyu.Han@uth.tmc.edu.
4Division of Child Psychiatry, Department of Psychiatry, University of Utah School of Medicine, Salt Lake City, UT 84108, USA. Electronic address: Amanda.Bakian@hsc.utah.edu.
5Division of Child Psychiatry, Department of Psychiatry, University of Utah School of Medicine, Salt Lake City, UT 84108, USA. Electronic address: Deborah.Bilder@hsc.utah.edu.
6Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA. Electronic address: rharrin5@jhu.edu.
7Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ 85721, USA. Electronic address: sydneyp@u.arizona.edu.
8Waisman Center, University of Wisconsin School of Medicine and Public Health, Madison, WI 53726, USA. Electronic address: mdurkin@wisc.edu.
9Department of Community and Family Health, College of Public Health, University of South Florida, Tampa, FL 33612, USA. Electronic address: rkirby@health.usf.edu.
10Department of Health Care Organization and Policy, School of Public Health, University of Alabama at Birmingham, Birmingham, AL 35205, USA.. Electronic address: mslay@uab.edu.
11National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA. Electronic address: bsr4@cdc.gov.
12Department of Pediatrics, Rutgers New Jersey Medical School, Newark, NJ 07103, USA. Electronic address: zahorodn@njms.rutgers.edu.

13Department of Psychiatry and Behavioral Sciences, University of Texas Medical School, Houston, TX 77054, USA. Electronic address: Deborah.A.Pearson@uth.tmc.edu.
14Division of Biostatistics, University of Texas School of Public Health at Houston, Houston, TX 77030, USA. Electronic address: Lemuel.A.Moye@uth.tmc.edu.
15National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA. Electronic address: xzb1@cdc.gov.

Abstract
Prenatal and perinatal exposures to air pollutants have been shown to adversely affect birth outcomes in offspring and may contribute to prevalence of autism spectrum disorder (ASD). **For this ecologic study, we evaluated the association between ASD prevalence, at the census tract level, and proximity of tract centroids to the closest industrial facilities releasing arsenic, lead or mercury** during the 1990s. We used 2000 to 2008 surveillance data from five sites of the Autism and Developmental Disabilities Monitoring (ADDM) network and 2000 census data to estimate prevalence. Multi-level negative binomial regression models were used to test associations between ASD prevalence and proximity to industrial facilities in existence from 1991 to 1999 according to the US Environmental Protection Agency Toxics Release Inventory (USEPA-TRI). Data for 2489 census tracts showed that after adjustment for demographic and socio-economic area-based characteristics, ASD prevalence was higher in census tracts located in the closest 10th percentile compared of distance to those in the furthest 50th percentile (adjusted RR=1.27, 95% CI: (1.00, 1.61), P=0.049). **The findings observed in this study are suggestive of the association between urban residential proximity to industrial facilities emitting air pollutants and higher ASD prevalence.**

106. Inflammatory Responses to Trivalent Influenza Virus Vaccine Among Pregnant Women

Vaccine. 2011 Nov 8;29(48):8982-7. doi: 10.1016/j.vaccine.2011.09.039. Epub 2011 Sep 22.

Christian LM, Iams JD, Porter K, Glaser R.

Department of Psychiatry, The Ohio State University Medical Center, Columbus, OH

Abstract
Objective
In the U.S., seasonal trivalent influenza vaccination (TIV) is currently universally recommended for all pregnant women. However, data on the maternal inflammatory response to vaccination is lacking and would better delineate the safety and clinical utility of immunization. In addition, for research purposes, vaccination has been used as a mild immune trigger to examine in vivo

inflammatory responses in nonpregnant adults. The utility of such a model in pregnancy is unknown. Given the clinical and empirical justifications, the current study examined the magnitude, time course, and variance in inflammatory responses following seasonal influenza virus vaccination among pregnant women.
Methods
Women were assessed prior to and at one day (n=15), two days (n=10), or approximately one week (n=21) following TIV. Serum interleukin (IL)-6, tumor necrosis factor (TNF)-α, C-reactive protein (CRP), and macrophage migration inhibitory factor (MIF) were determined by high sensitivity immunoassay.
Results
Significant increases in CRP were seen at one and two days post-vaccination (ps <.05). A similar effect was seen for TNF-α, for which an increase at two days post-vaccination approached statistical significance (p = .06). There was considerable variability in magnitude of response; coefficients of variation for change at two days post-vaccination ranged from 122% to 728%, with the greatest variability in IL-6 responses at this timepoint.
Conclusions
Trivalent influenza virus vaccination elicits a measurable inflammatory response among pregnant women. There is sufficient variability in response for testing associations with clinical outcomes. As adverse perinatal health outcomes including preeclampsia and preterm birth have an inflammatory component, a tendency toward greater inflammatory responding to immune triggers may predict risk of adverse outcomes, providing insight into biological mechanisms underlying risk. The inflammatory response elicited by vaccination is substantially milder and more transient than seen in infectious illness, arguing for the clinical value of vaccination. However, further research is needed to confirm that the mild inflammatory response elicited by vaccination is benign in pregnancy

107. Elevated maternal C-reactive protein and autism in a national birth cohort.

Mol Psychiatry. 2013 Jan 22. doi: 10.1038/mp.2012.197.

Brown AS, Sourander A, Hinkka-Yli-Salomäki S, McKeague IW, Sundvall J, Surcel HM.

Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York State Psychiatric Institute, New York, NY, USA, Department of Epidemiology, Columbia University Mailman School of Public Health, New York, NY, USA.

Abstract
Autism is a complex neuropsychiatric syndrome with a largely unknown etiology. Inflammation during pregnancy may represent a common pathway by which infections and other insults increase risk for the disorder. Hence, we investigated the association between early gestational C-reactive protein (CRP), an established inflammatory biomarker, prospectively assayed in maternal sera, and

childhood autism in a large national birth cohort with an extensive serum biobank. Other strengths of the cohort included nearly complete ascertainment of pregnancies in Finland (N=1.2 million) over the study period and national psychiatric registries consisting of virtually all treated autism cases in the population. Increasing maternal CRP levels, classified as a continuous variable, were significantly associated with autism in offspring. For maternal CRP levels in the highest quintile, compared with the lowest quintile, there was a significant, 43% elevated risk. This finding suggests that maternal inflammation may have a significant role in autism, with possible implications for identifying preventive strategies and pathogenic mechanisms in autism and other neurodevelopmental disorders.Molecular Psychiatry advance online publication, 22 January 2013; doi:10.1038/mp.2012.197.

108. What is regressive autism and why does it occur? Is it the consequence of multi-systemic dysfunction affecting the elimination of heavy metals and the ability to regulate neural temperature?

N Am J Med Sci. 2009 July; 1(2): 28–47.

Graham E. Ewing

Montague Healthcare, Nottingham United Kingdom

Abstract
There is a compelling argument that the occurrence of regressive autism is attributable to genetic and chromosomal abnormalities, arising from the overuse of vaccines, which subsequently affects the stability and function of the autonomic nervous system and physiological systems. That sense perception is linked to the autonomic nervous system and the function of the physiological systems enables us to examine the significance of autistic symptoms from a systemic perspective. Failure of the excretory system influences elimination of heavy metals and facilitates their accumulation and subsequent manifestation as neurotoxins: the long-term consequences of which would lead to neurodegeneration, cognitive and developmental problems. It may also influence regulation of neural hyperthermia. This article explores the issues and concludes that sensory dysfunction and systemic failure, manifested as autism, is the inevitable consequence arising from subtle DNA alteration and consequently from the overuse of vaccines.

109. Neurologic adverse events following vaccination

Prog Health Sci 2012, Vol 2 , No1

Sienkiewicz D.*, Kułak W., Okurowska-Zawada B., Paszko-Patej G.

Department of Pediatric Rehabilitation of the Medical University of Bialystok,

Poland

Abstract
The present review summarizes data on neurological adverse events following vaccination in the relation to intensity, time of onset, taking into account the immunological and non-immunological mechanisms. The authors described the physiological development of the immune system and the possible immune system responses following vaccination. Toxic property of thimerosal - a mercury-containing preservative used in some vaccines was presented. The neurological complications after vaccination were described. The role of vaccination in the natural course of infectious diseases and the current immunizations schedule in Poland was discussed.

Discussion by Sienkiewicz et. al.:
"**Among the "major" neurological complications, usually manifesting more than 48 hours after vaccination and which might be the cause of permanent damage to the central nervous system (CNS), the following are listed: seizures - especially if there is no increase in body temperature, hypotonic-hyporesponsive episodes, postvaccinal encephalitis, postvaccinal encephalopathy [6, 8-11] and autism [10, 12-14].**"

110. Immunological and autoimmune considerations of Autism Spectrum Disorders.

J Autoimmun. 2013 Jul 15. pii: S0896-8411(13)00073-5. doi: 10.1016/j.jaut.2013.05.005.

Gesundheit B, Rosenzweig JP, Naor D, Lerer B, Zachor DA, Procházka V, Melamed M, Kristt DA, Steinberg A, Shulman C, Hwang P, Koren G, Walfisch A, Passweg JR, Snowden JA, Tamouza R, Leboyer M, Farge-Bancel D, Ashwood P.

Jerusalem, Israel.

Abstract
Autism Spectrum Disorders (ASD) are a group of heterogeneous neurodevelopmental conditions presenting in early childhood with a prevalence ranging from 0.7% to 2.64%. Social interaction and communication skills are impaired and children often present with unusual repetitive behavior. The condition persists for life with major implications for the individual, the family and the entire health care system. While the etiology of ASD remains unknown, various clues suggest a possible association with altered immune responses and ASD. Inflammation in the brain and CNS has been reported by several groups with notable microglia activation and increased cytokine production in postmortem brain specimens of young and old individuals with ASD. Moreover several laboratories have isolated distinctive brain and CNS reactive antibodies from individuals with ASD. Large population based epidemiological studies have established a correlation between ASD and a family history of autoimmune diseases, associations with MHC complex haplotypes, and abnormal levels of

various inflammatory cytokines and immunological markers in the blood. In addition, there is evidence that antibodies that are only present in some mothers of children with ASD bind to fetal brain proteins and may be a marker or risk factor for ASD. Studies involving the injection of these ASD specific maternal serum antibodies into pregnant mice during gestation, or gestational exposure of Rhesus monkeys to IgG subclass of these antibodies, have consistently elicited behavioral changes in offspring that have relevance to ASD. We will summarize the various types of studies associating ASD with the immune system, critically evaluate the quality of these studies, and attempt to integrate them in a way that clarifies the areas of immune and autoimmune phenomena in ASD research that will be important indicators for future research.

111. Identification of Unique Gene Expression Profile in Children with Regressive Autism Spectrum Disorder (ASD) and Ileocolitis

PLoS ONE 8(3): e58058. doi:10.1371/journal.pone.0058058

Walker SJ, Fortunato J, Gonzalez LG, Krigsman A

Abstract
Gastrointestinal symptoms are common in children with autism spectrum disorder (ASD) and are often associated with mucosal inflammatory infiltrates of the small and large intestine. Although distinct histologic and immunohistochemical properties of this inflammatory infiltrate have been previously described in this ASDGI group, molecular characterization of these lesions has not been reported. In this study we utilize transcriptome profiling of gastrointestinal mucosal biopsy tissue from ASDGI children and three non-ASD control groups (Crohn's disease, ulcerative colitis, and histologically normal) in an effort to determine if there is a gene expression profile unique to the ASDGI group. Comparison of differentially expressed transcripts between the groups demonstrated that non-pathologic (normal) tissue segregated almost completely from inflamed tissue in all cases. Gene expression profiles in intestinal biopsy tissue from patients with Crohn's disease, ulcerative colitis, and ASDGI, while having significant overlap with each other, also showed distinctive features for each group. **Taken together, these results demonstrate that ASDGI children have a gastrointestinal mucosal molecular profile that overlaps significantly with known inflammatory bowel disease (IBD), yet has distinctive features that further supports the presence of an ASD-associated IBD variant, or, alternatively, a prodromal phase of typical inflammatory bowel disease.** Although we report qPCR confirmation of representative differentially expressed transcripts determined initially by microarray, these findings may be considered preliminary to the extent that they require further confirmation in a validation cohort.

112. Correlations between gene expression and mercury levels in blood of boys with and without autism.

Neurotox Res. 2011 Jan;19(1):31-48. doi: 10.1007/s12640-009-9137-7. Epub 2009 Nov 24.

Stamova B1, Green PG, Tian Y, Hertz-Picciotto I, Pessah IN, Hansen R, Yang X, Teng J, Gregg JP, Ashwood P, Van de Water J, Sharp FR.

Department of Neurology, University of California at Davis Medical Center, Sacramento, CA 95817, USA. boryana.stamova@ucdmc.ucdavis.edu

Abstract
Gene expression in blood was correlated with mercury levels in blood of 2- to 5-year-old boys with autism (AU) compared to age-matched typically developing (TD) control boys. This was done to address the possibility that the two groups might metabolize toxicants, such as mercury, differently. RNA was isolated from blood and gene expression assessed on whole genome Affymetrix Human U133 expression microarrays. Mercury levels were measured using an inductively coupled plasma mass spectrometer. Analysis of covariance (ANCOVA) was performed and partial correlations between gene expression and mercury levels were calculated, after correcting for age and batch effects. To reduce false positives, only genes shared by the ANCOVA models were analyzed. Of the 26 genes that correlated with mercury levels in both AU and TD boys, 11 were significantly different between the groups (P(Diagnosis*Mercury) ≤ 0.05). The expression of a large number of genes (n = 316) correlated with mercury levels in TD but not in AU boys (P ≤ 0.05), the most represented biological functions being cell death and cell morphology. Expression of 189 genes correlated with mercury levels in AU but not in TD boys (P ≤ 0.05), the most represented biological functions being cell morphology, amino acid metabolism, and antigen presentation. These data and those in our companion study on correlation of gene expression and lead levels show that AU and TD children display different correlations between transcript levels and low levels of mercury and lead. These findings might suggest different genetic transcriptional programs associated with mercury in AU compared to TD children.

113. Abnormal immune response to brain tissue antigen in the syndrome of autism.

Am J Psychiatry. 1982 Nov;139(11):1462-5.

Weizman A, Weizman R, Szekely GA, Wijsenbeek H, Livni E.

Abstract
Cell-mediated immune response to human myelin basic protein was studied by the macrophage migration inhibition factor test in 17 autistic patients and a control group of 11 patients suffering from other mental diseases included in the differential diagnosis of the syndrome of autism. Of the 17 autistic patients, 13

demonstrated inhibition of macrophage migration, whereas none of the nonautistic patients showed such a response. **The results indicate the existence of a cell-mediated immune response to brain tissue in the syndrome of autism**.

114. Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism.

Dig Dis Sci. 2000 Apr;45(4):723-9.

Kawashima H, Mori T, Kashiwagi Y, Takekuma K, Hoshika A, Wakefield A.

Department of Paediatrics, Tokyo Medical University, Japan.

Abstract
It has been reported that measles virus may be present in the intestine of patients with Crohn's disease. Additionally, a new syndrome has been reported in children with autism who exhibited developmental regression and gastrointestinal symptoms (autistic enterocolitis), in some cases soon after MMR vaccine. It is not known whether the virus, if confirmed to be present in these patients, derives from either wild strains or vaccine strains. In order to characterize the strains that may be present, we have carried out the detection of measles genomic RNA in peripheral mononuclear cells (PBMC) in eight patients with Crohn's disease, three patients with ulcerative colitis, and nine children with autistic enterocolitis. As controls, we examined healthy children and patients with SSPE, SLE, HIV-1 (a total of eight cases). RNA was purified from PBMC by Ficoll-paque, followed by reverse transcription using AMV; cDNAs were subjected to nested PCR for detection of specific regions of the hemagglutinin (H) and fusion (F) gene regions. Positive samples were sequenced directly, in nucleotides 8393-8676 (H region) or 5325-5465 (from noncoding F to coding F region). One of eight patients with Crohn disease, one of three patients with ulcerative colitis, and three of nine children with autism, were positive. Controls were all negative. The sequences obtained from the patients with Crohn's disease shared the characteristics with wild-strain virus. **The sequences obtained from the patients with ulcerative colitis and children with autism were consistent with being vaccine strains.** The results were concordant with the exposure history of the patients. Persistence of measles virus was confirmed in PBMC in some patients with chronic intestinal inflammation.

115. Mechanisms of aluminum adjuvant toxicity and autoimmunity in pediatric populations

Lupus. 2012 Feb;21(2):223-30. doi: 10.1177/0961203311430221.

L Tomljenovic, CA Shaw

Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada

Departments of Ophthalmology and Visual Sciences and Experimental Medicine and the Graduate Program in Neuroscience, University of British Columbia, Vancouver, BC, Canada

Lucija Tomljenovic, Post-doctoral fellow, Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia

Abstract
Immune challenges during early development, including those vaccine-induced, can lead to permanent detrimental alterations of the brain and immune function. Experimental evidence also shows that simultaneous administration of as little as two to three immune adjuvants can overcome genetic resistance to autoimmunity. In some developed countries, by the time children are 4 to 6 years old, they will have received a total of 126 antigenic compounds along with high amounts of aluminum (Al) adjuvants through routine vaccinations. According to the US Food and Drug Administration, safety assessments for vaccines have often not included appropriate toxicity studies because vaccines have not been viewed as inherently toxic. Taken together, these observations raise plausible concerns about the overall safety of current childhood vaccination programs. When assessing adjuvant toxicity in children, several key points ought to be considered: (i) infants and children should not be viewed as “small adults” with regard to toxicological risk as their unique physiology makes them much more vulnerable to toxic insults; (ii) in adult humans Al vaccine adjuvants have been linked to a variety of serious autoimmune and inflammatory conditions (i.e., “ASIA”), yet children are regularly exposed to much higher amounts of Al from vaccines than adults; (iii) it is often assumed that peripheral immune responses do not affect brain function. However**, it is now clearly established that there is a bidirectional neuro-immune cross-talk that plays crucial roles in immunoregulation as well as brain function. In turn, perturbations of the neuro-immune axis have been demonstrated in many autoimmune diseases encompassed in “ASIA” and are thought to be driven by a hyperactive immune response; and (iv) the same components of the neuro-immune axis that play key roles in brain development and immune function are heavily targeted by Al adjuvants.** In summary, research evidence shows that increasing concerns about current vaccination practices may indeed be warranted. Because children may be most at risk of vaccine-induced complications, a rigorous evaluation of the vaccine-related adverse health impacts in the pediatric population is urgently needed.

116. Etiology of autism spectrum disorders: Genes, environment, or both?

OA Autism 2014 Jun 10;2(2):11

C Shaw, S Sheth, D Li, L Tomljenovic
University of British Columbia, Vancouver, British Columbia, Canada

Introduction

Thus far, most of the research on both neurodevelopmental and neurodegenerative disorders has been focused on finding the presumed underlying genetic causes, while much less emphasis has been put on potential environmental factors. While some forms of autism are clearly genetic, the fact remains that heritability factors cannot adequately explain all reported cases nor their drastic increase over the last few decades. In particular, studies on twins have now shown that common environmental factors account for 55% of their risk for developing autism while genetic susceptibility explains only 37% of cases. Because the prenatal environment and early postnatal environment are shared between twins and because overt symptoms of autism emerge around the end of the first year of life, it is likely that at least some of the environmental factors contributing to the risk of autism exert their deleterious neurodevelopmental effect during this early period of life. Indeed, evidence has now emerged showing that autism may in part result from early-life immune insults induced by environmental xenobiotics. One of the most common xenobiotic with immuno-stimulating as well as neurotoxic properties to which infants under two years of age are routinely exposed worldwide is the aluminum (Al) vaccine adjuvant. In this review we discuss the mechanisms by which Al can induce adverse neurological and immunological effects and how these may provide important clues of Al's putative role in autism. Because of the tight connection between the development of the immune and the central nervous system, the possibility that immune-overstimulation in early infancy via vaccinations may play a role in neurobehavioural disorders needs to be carefully considered.

Conclusion
There is now sufficient evidence from both human and animal studies showing that cumulative exposure to aluminium adjuvants is not as benign as previously assumed. Given that vaccines are the only medical intervention that we attempt to deliver to every living human on earth and that by far the largest target population for vaccination are healthy children, a better appreciation and understanding of vaccine adjuvant risks appears warranted.

117. Thiol-modulated mechanisms of the cytotoxicity of thimerosal and inhibition of DNA topoisomerase II alpha.

Chem Res Toxicol. 2008 Feb;21(2):483-93.

Wu X, Liang H, O'Hara KA, Yalowich JC, Hasinoff BB.

Faculty of Pharmacy, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, R3T 2N2, Canada.

Abstract
Thimerosal is an organic mercury compound that is widely used as a preservative in vaccines and other solution formulations. The use of thimerosal has caused concern about its ability to cause neurological abnormalities due to mercury accumulation during a normal schedule of childhood vaccinations. While the chemistry and the biological effects of methylmercury have been well-studied, those of thimerosal have not. Thimerosal reacted rapidly with cysteine, GSH, human serum albumin, and single-stranded DNA to form ethylmercury adducts that were detectable by mass spectrometry. These results indicated that thimerosal would be quickly metabolized in vivo because of its reactions with protein and nonprotein thiols. **Thimerosal also potently inhibited the decatenation activity of DNA topoisomerase II alpha, likely through reaction with critical free cysteine thiol groups.** Thimerosal, however, did not act as a topoisomerase II poison and the lack of cross-resistance with a K562 cell line with a decreased level of topoisomerase II alpha (K/VP.5 cells) suggested that inhibition of topoisomerase II alpha was not a significant mechanism for the inhibition of cell growth. **Depletion of intracellular GSH with buthionine sulfoximine treatment greatly increased the K562 cell growth inhibitory effects of thimerosal, which showed that intracellular glutathione had a major role in protecting cells from thimerosal.** Pretreatment of thimerosal with glutathione did not, however, change its K562 cell growth inhibitory effects, a result consistent with the rapid exchange of the ethylmercury adduct among various thiol-containing cellular reactants. Thimerosal-induced single and double strand breaks in K562 cells were consistent with a rapid induction of apoptosis. In conclusion, these studies have elucidated some of the chemistry and biological activities of the interaction of thimerosal with topoisomerase II alpha and protein and nonprotein thiols and with DNA.

118. Topoisomerases facilitate transcription of long genes linked to autism

Nature (2013) doi:10.1038/nature12504
Received 17 January 2013 Accepted 24 July 2013 Published online 28 August 2013

Ian F. King, Chandri N. Yandava, Angela M. Mabb, Jack S. Hsiao, Hsien-Sung Huang, Brandon L. Pearson, J. Mauro Calabrese, Joshua Starmer, Joel S. Parker, Terry Magnuson, Stormy J. Chamberlain, Benjamin D. Philpot & Mark J. Zylka

Abstract
Topoisomerases are expressed throughout the developing and adult brain and are mutated in some individuals with autism spectrum disorder (ASD). However, how topoisomerases are mechanistically connected to ASD is unknown. Here we find that topotecan, a topoisomerase 1 (TOP1) inhibitor, dose-dependently reduces the expression of extremely long genes in mouse and human neurons, including nearly all genes that are longer than 200 kilobases. Expression of long genes is also reduced after knockdown of Top1 or Top2b in neurons, highlighting that both enzymes are required for full expression of long genes. By mapping

RNA polymerase II density genome-wide in neurons, we found that this length-dependent effect on gene expression was due to impaired transcription elongation. Interestingly, many high-confidence ASD candidate genes are exceptionally long and were reduced in expression after TOP1 inhibition. **Our findings suggest that chemicals and genetic mutations that impair topoisomerases could commonly contribute to ASD and other neurodevelopmental disorders.**

119. Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants, and autoimmunity.

Immunol Res. 2013 Jul;56(2-3):304-16.

Shaw CA, Tomljenovic L.

Abstract
We have examined the neurotoxicity of aluminum in humans and animals under various conditions, following different routes of administration, and provide an overview of the various associated disease states. The literature demonstrates clearly negative impacts of aluminum on the nervous system across the age span. In adults, aluminum exposure can lead to apparently age-related neurological deficits resembling Alzheimer's and has been linked to this disease and to the Guamanian variant, ALS-PDC. Similar outcomes have been found in animal models. In addition, injection of aluminum adjuvants in an attempt to model Gulf War syndrome and associated neurological deficits leads to an ALS phenotype in young male mice. In young children, a highly significant correlation exists between the number of pediatric aluminum-adjuvanted vaccines administered and the rate of autism spectrum disorders. Many of the features of aluminum-induced neurotoxicity may arise, in part, from autoimmune reactions, as part of the ASIA syndrome.

120. Behavioral abnormalities in young female mice following administration of aluminum adjuvants and the human papillomavirus (HPV) vaccine Gardasil.

Vaccine. 2016 Jan 8. pii: S0264-410X(16)00016-5. doi: 10.1016/j.vaccine.2015.12.067.

Inbar R1, Weiss R2, Tomljenovic L3, Arango MT4, Deri Y5, Shaw CA6, Chapman J7, Blank M5, Shoenfeld Y8.

Abstract
Vaccine adjuvants and vaccines may induce autoimmune and inflammatory manifestations in susceptible individuals. To date most human vaccine trials utilize aluminum (Al) adjuvants as placebos despite much evidence showing that

Al in vaccine-relevant exposures can be toxic to humans and animals. We sought to evaluate the effects of Al adjuvant and the HPV vaccine Gardasil versus the true placebo on behavioral and inflammatory parameters in young female mice. Six week old C57BL/6 female mice were injected with either, Gardasil, Gardasil+pertussis toxin (Pt), Al hydroxide, or, vehicle control in amounts equivalent to human exposure. At six months of age, Gardasil and Al-injected mice spent significantly more time floating in the forced swimming test (FST) in comparison to vehicle-injected mice (Al p=0.009; Gardasil, p=0.025; Gardasil+Pt, p=0.005). The increase in floating time was already highly significant at three months of age for the Gardasil and Gardasil+Pt group (p≤0.0001). No significant differences were observed in the number of stairs climbed in the staircase test nor in rotarod performance, both of which measure locomotor activity. Since rotarod also measures muscular strength, collectively these results indicate that differences observed in the FST were not due to locomotor dysfunction, but likely due to depression. Additionally, at three months of age, compared to control mice, Al-injected mice showed a significantly decreased preference for the new arm in the Y maze test (p=0.03), indicating short-term memory impairment. Moreover, anti-HPV antibodies from the sera of Gardasil and Gardasil+Pt-injected mice showed cross-reactivity with the mouse brain protein extract. Immunohistochemistry analysis revealed microglial activation in the CA1 area of the hippocampus of Gardasil-injected mice compared to the control. **It appears that Gardasil via its Al adjuvant and HPV antigens has the ability to trigger neuroinflammation and autoimmune reactions, further leading to behavioral changes.**

121. Transcriptomic Analyses of Neurotoxic Effects in Mouse Brain After Intermittent Neonatal Administration of Thimerosal.

Toxicol Sci. 2014 Apr 4.

Li X1, Qu F, Xie W, Wang F, Liu H, Song S, Chen T, Zhang Y, Zhu S, Wang Y, Guo C, Tang TS.

Abstract
Thimerosal is a vaccine antimicrobial preservative which has long been suspected an iatrogenic factor possibly contributing to neurodevelopmental disorders including autism. The association between infant vaccine thimerosal exposure and autism remains an open question. Although thimerosal has been removed from mandatory childhood vaccines in the United States, thimerosal-preserved vaccines are still widely used outside of the United States especially in developing countries. Notably, thimerosal-containing vaccines are being given to the newborns within the first 12-24 h after birth in some countries. To examine the possible neurotoxic effects of early neonatal exposure to a higher level of thimerosal, FVB mice were subcutaneously injected with thimerosal-mercury at a dose which is 20× higher than that used for regular Chinese infant immunization during the first 4 months of life. Thimerosal-treated mice exhibited neural development delay, social interaction deficiency, and inclination of depression.

Apparent neuropathological changes were also observed in adult mice neonatally treated with thimerosal. High-throughput RNA sequencing of autistic-behaved mice brains revealed the alternation of a number of canonical pathways involving neuronal development, neuronal synaptic function, and the dysregulation of endocrine system. Intriguingly, the elevation of anterior pituitary secreting hormones occurred exclusively in male but not in female thimerosal-treated mice, demonstrating for the first time the gender bias of thimerosal-mercury toxicity with regard to endocrine system. Our results indicate that higher dose of neonatal thimerosal-mercury (20× higher than that used in human) is capable of inducing long-lasting substantial dysregulation of neurodevelopment, synaptic function, and endocrine system, which could be the causal involvements of autistic-like behavior in mice.

122. Self-organized criticality theory of autoimmunity.

PLoS One. 2009 Dec 31;4(12):e8382. doi: 10.1371/journal.pone.0008382.

Tsumiyama K1, Miyazaki Y, Shiozawa S.

Department of Biophysics, Kobe University Graduate School of Health Science, Kobe, Japan.

Abstract
BACKGROUND:
The cause of autoimmunity, which is unknown, is investigated from a different angle, i.e., the defect in immune 'system', to explain the cause of autoimmunity.

METHODOLOGY/PRINCIPAL FINDINGS:
Repeated immunization with antigen causes systemic autoimmunity in mice otherwise not prone to spontaneous autoimmune diseases. Overstimulation of CD4(+) T cells led to the development of autoantibody-inducing CD4(+) T (aiCD4(+) T) cell which had undergone T cell receptor (TCR) revision and was capable of inducing autoantibodies. The aiCD4(+) T cell was induced by de novo TCR revision but not by cross-reaction, and subsequently overstimulated CD8(+) T cells, driving them to become antigen-specific cytotoxic T lymphocytes (CTL). These CTLs could be further matured by antigen cross-presentation, after which they caused autoimmune tissue injury akin to systemic lupus erythematosus (SLE).

CONCLUSIONS/SIGNIFICANCE:
Systemic autoimmunity appears to be the inevitable consequence of over-stimulating the host's immune 'system' by repeated immunization with antigen, to the levels that surpass system's self-organized criticality.

123. Can Awareness of Medical Pathophysiology in Autism Lead to Primary Care Autism Prevention Strategies?

Elizabeth Mumper, MD, FAAP

N A J Med Sci. 2013;6(3):134-144. DOI: 10.7156/najms.2013.0603134

Abstract
Emerging research suggests that the timing of environmental factors in the presence of genetic predispositions has influenced the increase in autism spectrum disorders over the past several decades. A review of the medical literature suggests that autism may be impacted by environmental toxicants, breastfeeding duration, gut flora composition, nutritional status, acetaminophen use, vaccine practices and use of antibiotics and/or frequency of infections. The author reports her retrospective clinical research in a general pediatric practice (Advocates for Children), which shows a modest trend toward lower prevalence of autism than her previous pediatric practice or recent CDC data. Out of 294 general pediatrics patients followed since 2005 there were zero new cases of autism (p value 0.014). Given the prevalence of autism for that cohort of 1 in 50 children in the United States, it is important to consider implementing strategies in primary care practice that could potentially modify environmental factors or affect the timing of environmental triggers contributing to autism.

124. Autistic disturbances of affective contact.

Nervous Child 2, 217-250 (1943)

Kanner L.
Johns Hopkins University

“Case 3. Richard M. was referred to the Johns Hopkins Hospital on February 5, 1941, at 3 years, 3 months of age, with the complaint of deafness because he did not talk and did not respond to questions.”

“**Following smallpox vaccination at 12 months, he had an attack of diarrhea and fever, from which he recovered in somewhat less than a week**.”

“In September, 1940, the mother, in commenting on Richard's failure to talk, remarked in her notes: I can't be sure just when he stopped the imitation of words sounds. It seems that he has gone backward mentally gradually for the last two years.”

Richard M:

November 1937 – Born

November 1938 – Vaccinated with Smallpox vaccine

September 1940 – Mother reports developmental regression beginning approximately two years previously

February 1941 – Referred to Hopkins for evaluation

1943 – Becomes the third child to be described as autistic by Leo Kanner in his disorder defining paper, the first paper published on autism.